VOL. 57 FASC 1

acta ophthalmologica

A K. K. K. LUNDSGAARD EDI COEPTA

SCRIPTOR COPENHAGEN FEBRUARY 1979

ISSN 0001-639 X

acta
ophthalmologica

Instructions to Contributors

Manuscripts from Scandinavian countries should be sent to the Chief Editor

From other countries to an International Editor

Acta Ophthalmologica publishes articles dealing with clinical and basic ophthalmology *Priority is given to manuscripts which are original brief and precise Papers are accepted on the condition* that they have not and will not be published elsewhere except under certain circumstances as when a paper has been read before a society The author must then indicate the name of the society and date of the meeting It is assumed that the author has considered the ethical aspects of his research and in this connection paid attention to the guidelines of the Helsinki declaration as revised in 1975 Articles including references must not exceed 10 printed pages One printed Acta page contains about 3150 letters space between words included Additional pages when accepted must be paid for by the author at a rate of D kr 250 per page

MANUSCRIPTS must be submitted in duplicate to facilitate rapid refereeing They should be typewritten on one side of the paper with double spacing and a wide margin The manuscript should include a title page abstract, key words references tables and legends The author should retain a copy for his own use Manuscripts are not insured against loss or damage

The manuscript should be arranged in the following order 1) title page 2) abstract, 3) key words 4) text, 5 acknowledgment, 6) references 7) author s address 8) tables 9) legends for figures

Manuscripts must be submitted on standard sized manuscript paper Illustrations and tables should not exceed this size and should be enclosed with the manuscripts

THE TITLE PAGE should contain on separate lines the author(s) institutions and the head(s) of the department, the title of the article and the name(s) of the author(s) A short title not exceeding 40 letters and spaces will be required for use as a running head and should be written at the bottom of the title page

THE ABSTRACT *should not exceed 150 words and is placed before the text. Reproduction of authors* abstracts is authorized

KEY WORDS Four to nine key words for indexing purposes should be given

TEXT Papers should be clearly divided into the following sections introduction material methods results and findings, discussion. Introduction the nature of the problem present knowledge and purpose of the article Extensive literature reviews are rarely desirable Primary sources are preferred Material on which the observations were made Methods of investigation with sufficient information to permit others to repeat the observations. Results should be supported by statistical analysis when necessary Discussion should be based directly on the author(s) contributions within the framework of prior investigations Unsupported hypotheses should be avoided

Every page of the manuscript must be marked with the running head including the name of the

(Cont. cover page 3)

acta ophthalmologica

A K K.K LUNDSGAARD EDI COEPTA

VOLUME 57

Scriptor Copenhagen 1979

Printed in Denmark
by Bogtrykkeriet Forum
Copenhagen

AUTHORS INDEX

JUDICIA DE NOVIS LIBRIS

SUPPLEMENTA

VARIA

SUBJECT INDEX

Department of Ophthalmology Rigshospitalet Copenhagen
(Heads V Dreyer J Edmund E Gregersen S V Kessing and H H Seedorff)

VARIATIONS OF LENS THICKNESS IN RELATION TO BIOMICROSCOPIC TYPES OF HUMAN SENILE CATARACT

BY

A. BRUUN LAURSEN and H FLEDELIUS

The results are reported of 38 ultrasonographic *in vivo* mensurations of intraindividual differences in axial thickness between a cataractous lens in one eye and a biomicroscopically clear or slightly cataractous lens (incipient deep cortical opacity) in the other Obviously intumescent cataractous lenses were excluded
In general the cataractous lens was *thinner* than the contralateral clear or slightly cataractous lens
Large decreases in lens thickness appeared in lenses with the *capsule near opacities* of posterior subcapsular cataract (PSC) + anterior capsular/subcapsular opacity (ACSCO) PSC was more closely correlated to lens thinning than was ACSCO
Nuclear cataract very often occurred in thin lenses but did not appear to cause lens thinning *per se*
Deep cortical opacity was not associated with lens thinning
The present results contributed to our argumentation that the decrease in lens thickness is due to a leak of lens material through the lens membrane beside a possible cessation of growth of the lens fibres

Key words cataract unilateral human senile – biomicroscopy – ultrasonography – lens thickness intraindividual side differences in – opacities capsule near

It is the aim of the present study to evaluate possible associations between biomicroscopical types of opacities in human senile cataractous lenses and ultrasonographically recorded variations in lens thickness (LT) In particular

Received August 15 1978

we were interested in clarifying whether anterior capsular/subcapsular opacity (ACSCO) – Bruun Laursen 1976 see Methods below – might be associated with LT changes since ACSCO seems to be a *biomicroscopical* indicator of important *biochemical* changes during senile cataractogenesis in man. Thus ACSCO in human senile cataract has been found to be associated with a) low ribonucleotide pools (Bruun Laursen 1976) b) an increase in lens sodium ion concentration and c) a fall in lens potassium ion concentration (Klauber & Bruun Laursen 1977)

The obvious *macroscopical* characteristic of the cataractous lens is of course its opacification but in addition many cataractous lenses are *smaller* (less voluminous) than clear lenses of coeval persons, as was first shown by Sm..h (1883) More recently this has been confirmed through investigations into lens wet weights (Maraini & Mangili 1973) and by *in vivo* ultrasonic lens thickness mensurations (Babel et al 1969 Delmarcelle & Luyckx Bacus 1971) The latter authors further included optical pachymetry of the depth of the anterior chamber and roughly examined its possible relations to biomicroscopical types of cataract. They found that the thinnest lenses and the deepest chambers occurred when the lenses were totally opaque.

To our knowledge there have been no reports on the topic dealt with in the present paper The possible associations between echographically recorded variations in ΔLT (the intraindividual side difference in lens thickness in persons with unilateral cataract) and specified cataract morphology

Material

58 consecutive patients aged 51–83 years with predominantly unilateral senile cataract were selected for the study Excluded were patients with previous eye injuries or additional eye disease diabetes mellitus as well as patients receiving corticosteroid treatment. Excluded were also obvious cases of intumescent (Morgagni) cataract. These exclusions imply that our statements will be valid for non intumescent cataracts only and not for the *full* spectrum of human senile cataracts

We intended to include only strictly unilateral cases of senile cataract but such a material is difficult to collect considering the above criteria of exclusion as well as the age of the patients Even in a fellow eye with a visual acuity of 1 0 careful examination will as a rule reveal lens opacities in the age group under study There were however 24 cases of *strictly unilateral* senile cataract. The remaining 14 cases presented marked side differences concerning cataract morphology In particular we demanded that PSC and ACSCO be absent in the less affected lens In general this was affected by incipient deep cortical opacity only

The axial lengths of the eyes were used to exclude a possible source of error in the evaluation of ΔLT namely a pre existing anisometropia, now concealed behind

opaque media In fact, the axial lengths within the 38 pairs of eyes were almost identical the median *side difference* being only 0 2 mm and in all but 2 cases ≦ 0 4 mm

Methods

Three classification systems were used (Bruun Laursen) based on slit lamp examination and ophthalmoscopy according to a) *Site(s) of opacity* within the lens b) *Extent of PSC (posterior subcapsular cataract) and ACSCO* c) An overall impression of the *transparency* of the lens

a) *Sites of opacity*

1) Anterior capsular/subcapsular opacity (ACSCO) whitish dots or an irregular greyish coating apparently located in or immediately beneath the anterior lens capsule (Bruun Laursen 1976)

2) Posterior subcapsular cataract (PSC) posterior subcapsular tufaceous opacity

3) Nuclear cataract grey or brown opacity of the biomicroscopic lens nucleus causing blurred insight to the posterior lens capsule and appearing as a disciform opacity on transillumination with the ophthalmoscope (mydriasis)

4) Cortical cataract irregular or spoke like anterior or posterior *deep* cortical opacities apparently free of the lens capsule

5) Totally opaque lenses uniformly grey or brownish lenses always with extensive ACSCO

b) *Extent of PSC and ACSCO* estimated as percentages of the visible parts of the anterior and posterior lens surfaces respectively

c) *Lens transparency* as estimated roughly on slit lamp and ophthalmoscopic examinations was recorded as

Grade 0 quite clear

Grade 1 a high degree of transparency and a comparatively slight extension of the opacity

Grade 2 more severely affected immature cataract

Grade 3 total lens opacity

The above evaluations were just as the following ultrasound mensurations performed in full mydriasis after instillation of phenylephrine 10 % and cyclopentolate 0 5 % or tropicamide 1 %

Axial ultrasound measurements were performed with A mode technique as described by Fledelius (1976) and they were all carried out by him Kretztechnik 7000 a 10 Mc ultrasonolux transducer and a Methocel filled contact glass were used

For the conversion from arbitrary apparatus units to lens thickness in mm we used the intralenticular ultrasound velocity of 1641 m/sec given by Jansson & Kock (1962) for *clear* lenses The velocity in cataractous lenses has been found to be of the same order 1629 m/sec (Coleman et al 1975) The aberration being less than 1 per cent we did not introduce this latter value in calculations for the cataractous lenses

A pilot study was performed by Fledelius (quoted by Fledelius & Bruun Laursen 1978) 1) to control the US method and 2) to estimate the level of intraindividual

LT differences (right left) in a sample with healthy eyes ΔLT values were – as expected – low with a median value ≤ 0 1 mm

Statistical comparisons between two types of lens opacity were carried out by means of the Mann Whitney test and correlation analyses by means of the Spearman rank correlation analysis

Table I

Intraindividual side differences in lens thickness (ΔLT) between a cataractous and a clear lens in 24 persons arranged according to various biomicroscopic types The individual opacities were compiled regardless of additional opacities in the same lenses In a few cases the posterior cortex and the posterior capsule of immature cataractous lenses could not be evaluated *Abbreviations* PSC posterior subcapsular cataract ACSCO anterior capsular/subcapsular opacity LT = lens thickness See Methods

Type of opacity	Median ΔLT mm	Range ΔLT mm	LT of cataractous lenses		Median age years	N
			Median mm	Range mm		
+PSC+ACSCO	-1 0	-0 4 – -1 6	4 0	3 6 – 4 1	68	5
+PSC	-0 5	-0 2 – -1 6	4 1	3 6 – 4 5	64	8
+ACSCO	-0 4	-0 1 – -1 6	4 0	3 6 – 4 8	68	
–ACSCO	-0 25	0 – -0 6	4.5	3 9 – 5 1	64 5	10
–PSC	-0 1	0 – -0 6	4 6	3 9 – 5 1	6 5	8
–PSC–ACSCO	-0 1	0 – -0 6	4 5	3 9 – 5 1	66	
Nuclear opacity	-0 4	-0 1 – -1 6	4 0	3 6 – 4 8	60	5
–Nuclear opacity	-0 3	0 – -0 6	4 4	3 9 – 5 1	64	9
Deep cortical opacity	-0 3	0 – -1 6	4 5	3 6 – 5 1	6	12
–Deep cortical opacity	-0 4	-0 2 – -1 0	4 3	4 0 – 4 5	60	5
Lens transparency						
Grade 1	0 2	0 – -0 6	4 5	3 9 – 5 1	65	11
Grade 2	0	-0 3 – -1 6	4 0	3 6 – 4 8	65 5	6
Grade 3 (total opacity)	-0	-0 4 – -1 4	3 9	3 6 – 4 6	65	

Results

In the following the results will be viewed from two aspects.

First the intraindividual side differences in axial lens thickness (ΔLT) will be given in relation to different types of lens opacity. This is done a) for the strictly unilateral cases (N = 24) Table I, Fig. 1) and b) for the combined group of unilateral + bilateral cases (N = 38, Table II, Figs. 2 and 3).

Besides, the frequencies of the different kinds of lens opacity in immature cataractous lenses are shown in relation to intraindividual side differences in lens thickness (Tables III and IV), the material being roughly divided into a group of numerically high and a group of numerically low ΔLT values.

Table II

Differences in intraindividual lens thickness (ΔLT) between a cataractous lens on one side and a clear or slightly cataractous lens (with only incipient deep cortical opacity) on the other side, again arranged according to biomicroscopic types. N = 38. See legend of Table I also (1 lens was inadvertently not graded).

Type of opacity	Median ΔLT mm	Range ΔLT mm	LT of cataractous lenses		Median age years	N
			Median mm	Range mm		
PSC+ACSCO	-0.75	-0.4 – -1.6	3.9	3.4–4.6	0	10
+PSC	-0.6	-0.2 – -1.6	4.0	3.4–4.6	67	12
ACSCO	-0.65	-0.1 – -1.6	4.0	3.4–4.8	70	12
-ACSCO	-0.25	0 – -1.1	4.4	3.5–5.1	65.5	12
-PSC	-0.1	0 – -0.6	4.5	3.9–5.1	69	9
-PSC-ACSCO	-0.15	0 – -0.6	4.5	3.9–5.1	67.5	8
Nuclear opacity	-0.6	-0.1 – -1.6	4.0	3.4–4.8	70	13
-Nuclear opacity	-0.25	0 – -0.6	4.4	3.9–5.1	65	10
Lens transparency						
Grade 1	-0.2	0 – -0.6	4.5	3.9–5.1	65.5	12
Grade 2	-0.8	-0.3 – -1.6	4.0	3.4–4.8	72	11
Grade 3 (total opacity)	-0.6	+0.1 – -1.4	4.0	3.2–4.6	69	14

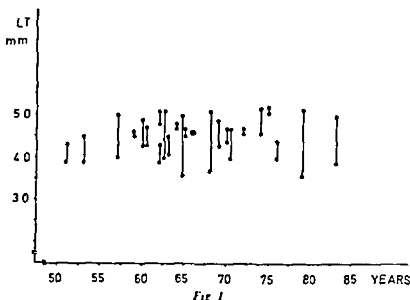


Fig 1

Ultrasonographically recorded intraindividual side differences in lens thickness (ΔLT) between a cataractous and a clear lens (N = 24) in relation to age. No significant correlation was found between age and the thickness of the clear lenses in the present age interval of 51–83 years (Spearman's rank correlation coefficient corrected for ties = 0.38; $0.10 > P > 0.05$). ○ clear lens ● cataractous lens

The ΔLT values will be given with signs as the LT value of the cataractous lens minus the LT value of the clear(er) lens. In most cases the sign is a minus, to signify a deficit in the thickness of the cataractous as compared with the clear(er) lens.

The various lens opacities were of course mingled in many combinations in our material, and it is therefore difficult to evaluate the association between the separate opacity and the corresponding ΔLT. However, this is attempted in Tables I–IV, in Figs 2 and 3, as well as in our statistical calculations. Thus the ΔLT value of an immature cataractous lens with PSC (posterior subcapsular cataract), ACSCO (anterior capsular/subcapsular opacity), cortical *and* nuclear opacities was used 5 times, namely each time each separate opacity was considered regardless of the 3 additional opacities and when the lens transparency was graded.

Fig 1 shows the individual lens thicknesses (LT) of both eyes of the 24 patients with strictly unilateral cataract. A slight increase in LT with age possibly occurred for the clear lenses. However, the significance level was only $0.10 > P > 0.05$.

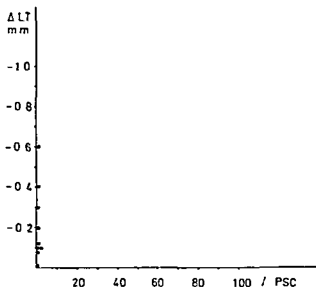


Fig 2

The correlation between the extent of PSC (posterior subcapsular cataract) and ΔLT i e the intraindividual difference in lens thickness between an immature cataractous lens on one side and a clear or slightly cataractous lens with incipient deep cortical opacity on the other side Spearman s rank correlation coefficient uncorrec ed for ties = 0 81 $P < 0\ 001$ N = 19 5 lenses were excluded (compare Table II) because the extent of PSC could not be evaluated

Site of opacity degree of opacification

From Table I comprising the group of strictly unilateral cataracts it is obvious that the numerically highest median ΔLT values i e the thinnest lenses are associated *with* PSC + ACSCO (–1 0 mm $P < 0\ 02$) *with* PSC (–0 5 mm $P < 0\ 02$) and *with* grade 2 and 3 cataract (–0 4 mm in both instances $P < 0\ 05$ and $P < 0\ 01$ respectively) The P values refer to com parisons with lenses *without* PSC or ACSCO *without* PSC and to grade 1 lenses respectively

The ΔLT values of immature cataractous lenses *with* ACSCO did not differ from those of immature cataractous lenses *without* this opacity Nor was there any statistical difference in the strictly unilateral cases as for ΔLT values between immature cataractous lenses with and without nuclear opacity or with and without deep cortical opacity

Isolated deep cortical cataract – the type that occurred in the clearer lens of the almost unilateral cases – presented *low* ΔLT values the median ΔLT value for pure cortical grade 1 lenses being –0 1 mm (range = 0 – –0 6

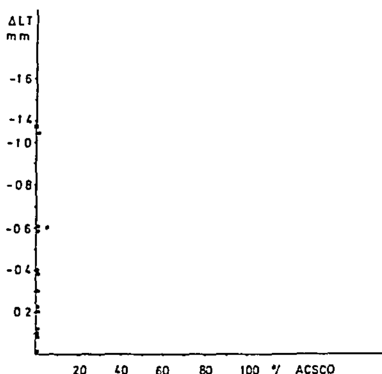


Fig 3
The correlation between the extent of ACSCO (anterior capsular/subcapsular opacity) and ΔLT (see legend Fig 2) Spearman's rank correlation coefficient uncorrected for ties = 0.59 0.01 > P > 0.001 N = 24

Table III
Distribution of various biomicroscopic lens opacities in numerically high (≥ 0 6 mm) and low (≤ 0 4 mm) values of intraindividual side differences in lens thickness (ΔLT) between an immature cataractous lens and a clear lens in 17 persons See Legend of Table I also Median ΔLT = -0 4 mm

ΔLT mm	PSC ACSCO	PSC	ACSCO	nuclear opacity	+deep cortical opacity	Grade I (grades 1+2 100%)	N
≥ 0 6	60%	80%	60%	60%	60%	40%	5
≤ 0 4	18%	36%	33%	42%	5%	75%	12

Table IV

Distribution of various biomicroscopic lens opacities in nummerically high (≧ 0 6 mm) and low (≦ 0 4 mm) values of ΔLT between an immature cataractous and a clear or slightly cataractous lens with incipient deep cortical cataract N = 24 See lengend of Table I also Median ΔLT = –0 4 mm

ΔLT mm	+PSC+ ACSCO	+PSC	+ACSCO	+Nuclear opacity	Grade 1 (grades 1+2 = 100 %)	N
≧ 0 6	73 %	90 %	3 %	82 %	90 %	11
≦ 0 4	17 %	33 %	31 %	38 %	77 %	13

mm not specified in able I) On this background it was considered justifiable to pool the strictly and the almost unilateral cases and the combined results are presented in Table II

The significant differences appearing after statistical evaluation of the material presented in Table I reappear for the findings presented in Table II Besides the induction in Table II of the 14 almost unilateral cases resulted in additional statistically significant ΔLT differences Thus immature cataractous lenses *with* PSC + ACSCO (–0 75 mm $P < 0\,01$) *with* PSC (–0 6 mm $P < 0\,01$) *with* ACSCO (–0 65 mm $P < 0\,02$) *with* nuclear opacity (–0 6 mm $P < 0\,02$) *with* grade 2 cataract (–0 8 mm $P < 0\,01$) and lenses *with* grade 3 cataract (–0 6 mm $P < 0\,05$) had numerically higher ΔLT values – i e they were more thinned – than had lens pairs without these opacities Again for grade 2 and 3 lenses the P values refer to comparisons with the ΔLT values of grade 1 lenses

In the combined group the ΔLT values of immature cataractous lenses *with* PSC + ACSCO PSC ACSCO nuclear cataract or grade 2 cataract did not differ significantly from the ΔLT values of totally opaque (= grade 3) lenses The above characteristics are also obvious in Tables III and IV which are divided by the size of ΔLT Again lenses *with* PSC + ACSCO PSC ACSCO nuclear opacity or grade 2 cataract dominate in the numerically high ΔLT group (higher degree of lens thinning) and correspondingly they are rare in the numerically low ΔLT group Cortical cataract appears equally often in both groups (Table III)

ACSCO has not been observed as an *isolated* apacity by the authors neither in this material nor in other patients from the Eye Clinic In particular it is often combined with PSC out of 11 immature cataractous lenses with ACSCO in which the posterior capsule could also be estimated 10 had PSC

As far as the *opacification* of the immature cataractous lenses is concerned nuclear cataract appeared to be more important than did deep cortical opacity Thus nuclear cataract was found in only 2 out of 12 grade 1 lenses in contrast with 11 out of 11 grade 2 lenses (1 lens was inadvertently not graded) Among the latter 11 lenses a deep cortical cataract was unmistakable in 5 slight only in 2 and absent in 4

Extent of PSC and ACSCO

Correlation analysis confirmed the aforementioned results Positive correlations were found for the *extents* of PSC and ACSCO on the one hand and the numerical *size of* ΔLT on the other This means that the cataractous lenses were more thinned the higher the percentages of PSC and ACSCO were found to be (Figs 2 and 3)

Discussion

Associations between degree of lens thinning and biomicroscopic types of cataract

PSC and ACSCO appeared to be the most important opacities in this context Above all a high positive correlation was found between the *extent* of PSC *and the decrease in lens thickness Next ACSCO also showed a* definitely positive correlation to the decrease in lens thickness but this may in part at least be due to the common occurrence of PSC in lenses with ACSCO Thus the ΔLT *differences* between strictly unilateral cataracts with and without ACSCO proved to be insignificant

Lens thinning was more pronounced in grade 2 cataracts *(all of which* presented nuclear opacity) than in grade 1 cataracts which rarely presented nuclear opacity This suggests an association of nuclear opacity with lens thinning However the following observation indicates that nuclear cataract does not cause lens thinning *per se* The ΔLT *difference* between lenses with and without nuclear opacity was insignificant in the strictly unilateral cases

Keeping constantly in mind the hazards connected with an approach so multifactorial as ours we feel able to state that deep cortical opacity was not associated with the process of lens thinning

Does a cessation of lens fibre growth or a real decrease in thickness of the cataractous lens account for the side difference in lens thickness?

Biomicroscopically the smaller lens of cataract has been ascribed to a thinning of the lens cortex (Goldmann & Favre 1961 Brown 1973) while the

thickness of the nucleus of senile cataractous lenses appears to remain constant (Rodriguez Caballero et al 1973) The reason for the thinning of the cata ractous lens has been claimed to be a cessation of the (ordinarily continuous) growth of the lens fibres (Goldmann & Niesel 1964) possibly setting in even before lens opacities become visible (Delmarcelle & Luyckx Bacus 1971)

Our study does *not* support the theory of a cessation of lens fibre growth at least not as the only or main reason for the thinning of the cataractous lens Clear lenses thicken 0 23 mm per decade in the age interval of 20–60 years (Weekers et al 1973) and probably at a somewhat slower rate after the age of 60 years Provided that the theoretic cessation of lens fibre growth was the only reason for lens thinning some of our cataractous lenses should have stopped growing – in one eye only – already in early adolescence or childhood Or for our extreme ΔLT values even *before* birth!

Furthermore the so called normal *fellow* eye in cases of unilateral pre senile/senile cataract frequently develops a similar cataract (with PSC and/or ACSCO) in the course of a few years In other words the cataract of the first affected eye often remains unilateral for a limited period of time only In such cases an intraindividual side difference in lens thickness – ΔLT – should in fact be minimal or absent if the cessation of growth theory were the only valid one The high ΔLT values of the present material indicate therefore that the thinning of the cataractous lens cannot be accounted for by a precocious arrest of lens fibre growth only

All things considered our carefully selected material of largely unilateral cataracts – the fellow eye serving as a control – strongly suggests a *real decrease in lens thickness* in some biomicroscopical types of human senile cata ract In our opinion this is the most fruitful working hypothesis for further research

Is the decrease in lens thickness caused by a leak of lens substance into the aqueous?

The most likely account for a decrease in lens size is an escape of lens material through a lens membrane The membranes of the lens hypothetically comprise the capsule + the epithelium anteriorly and the lens capsule poste riorly In this context we would like to draw attention to Friedenwald s (1930) studies This author found evidence of an increase in permeability of the lens capsule after prolonged exposure of it to cataractous lens cortex Later it has been suggested that proteins of low molecular weight leak from the lens to the aqueous during senile cataractogenesis (Mach 1963 Charlton & van Hey ningen 1968 van Heyningen 1972) In accordance with this Klauber & Bruun

Laursen (1977) found low dry weight (= protein) percentages in totally opaque lenses Since such lenses are small as compared with e g the thicker immature cataractous lenses totally opaque lenses obviously contain less protein than do thick immature cataractous lenses

We find it probable that the capsule near opacities of ACSCO and in particular PSC (only a single barrier posteriorly the lens capsule) may be associated with an increase in lens membrane permeability To our knowledge however no studies have been carried out so far on this topic

Concluding Remarks

In summary our concepts on the decrease in thickness of the biomicroscopically prevailing types of human senile cataract (excluding intumescent lenses) are as follows

1) In most cases the cataractous lens is definitely *thinner* than the clear lens of the fellow eye

2) The intraindividual side difference in lens thickness (ΔLT) is associated with PSC (in particular) and ACSCO and – to a minor extent – with nuclear opacity Deep cortical cataract appears not to be associated with lens thinning

3) The ΔLT is probably accounted for mainly by a real decrease in lens thickness and *not only* by a cessation of lens fibre growth

4) The decrease in lens thickness may be accounted for by a leak of lens material through the lens membranes

5) This leak of lens substance appears to be associated with the occurrence of the *capsule near opacities* (PSC and ACSCO)

References

Bal el J Isilas K & Itin W (19C9) Mesures échographiques de l'épaisseur du cristallin dans les cataractes unilatérales In Bock J and Ossoinig K Eds *Ultrasonographia Medica Vol* ? pp 547–555 Verlag der Wiener Medizinischen Akademie Vienna (SIDUO III)

Brown N A P (19 3) General discussion I Lens change with age and cataract Slit image photography In Ciba Foundation Symposium 19 (new series) Ed *The human lens in relation to cataract* pp 65–78 Associated Scientific Publishers Amsterdam London New York

Bruun Laursen A (19 6) Concentrations of some ribonucleotides L lactate and pyruvate in human senile cataractous lenses with special reference to anterior capsular/subcapsular opacity *Acta ophthal (Kbh)* 54 647–69?

Charlton J & van Heyningen R (1968) An investigation into the loss of proteins of low molecular size from the lens in senile cataract *Exp Eye Res* 7 4-55
Coleman D J Lizzi F L Franzen L A & Abramson D M (1975) A determination of the velocity of ultrasound in cataractous lenses In Francois J and Goes F Eds *Ultrasonography in Ophthalmology Bibl Ophthal 83* 246-251 Basel
Delmarcelle Y & Luyckx Bacus J (1971) Biometrie du segment anterieur dans la cataracte senile *Acta ophthal (Kbh) 49* 454-466
Fledelius H (1976) Prematurity and the Eye Ophthalmic 10 year follow up of children of low and normal birth weight *Acta ophthal (Kbh) Suppl 128* 35-46
Fledelius H & Bruun Laursen A (1978) *On lens thickness in cataractous lenses* Read at the SIDUO VII Congress Munster Sept 1978
Friedenwald J S (1930) Permeability of the lens capsule with special reference to the etiology of senile cataract *Arch Ophthal (Chicago) 3* 182-193
Goldmann H & Favre M (1961) Eine besondere Form praseniler Katarakt *Ophthalmologica 141* 418-422
Goldmann H & Niesel P (1964) Studien uber Abspaltungsstreifen und das Linsen wachstum *Ophthalmologica 147* 134-147
Jansson F & Kock E (1962) Determination of the velocity of ultrasound in the human lens and vitreous *Acta ophthal (Kbh) 40* 420-433
Klauber A & Bruun Laursen A (19) Relative contents of sodium potassium, water and dry matter in human senile cataractous lenses in relation to anterior capsular/subcapsular opacity *Acta ophthal (Kbh) 55* 189-199
Mach H (1963) Untersuchungen von Linseneiweiss und Mikroelektrophorese von was serloslichem Eiweiss in Altersstar *Klin Mbl Augenheilk 143* 689-710
Maraini G & Mangili R (1973) Differences in proteins and in the water balance of the lens in nuclear and cortical types of senile cataract In Ciba Foundation Symposium 19 (new series) Ed *The human lens – in relation to cataract* pp 79-97 Associated Scientific Publishers Amsterdam London New York
Rodriguez Caballero M L Gerhard J P & Nordmann J (1973) Le noyeau du cristallin humain II Son epaisseur *Arch Ophthal (Paris) 33* 425-428
Smith P (1883) Diseases of crystalline lens and capsule *Trans ophthal Soc U.K 3* 79-99
van Heyningen R (1972) The human lens I A comparison of cataracts extracted in Oxford (England) and Shikarpur (W Pakistan) *Exp Eye Res 13* 136-147
Weekers R Delmarcelle Y Luyckx Bacus J & Collignon J (1973) Morphological changes of the lens with age and cataract In Ciba Foundation Symposium 19 (new series) Ed *The human lens – in relation to cataract* pp 25-40 Associated Scientific Publishers Amsterdam London New York

Authors addresses

A Bruun Laursen
Eye Department 539
Hvidovre Hospital
Kettegård Alle 30
2650 Hvidovre
Denmark

H Fledelius
Eye Department 2061
Rigshospitalet
Blegdamsvej 9
2100 Copenhagen Ø
Denmark

Department of Ophthalmology (Head \ Ehlers)
Ārhus Kommunehospital
and Institute of Anatomy B (Former head Th Blackstad)
University of Aarhus Arhus Denmark

ANT EGG CATARACT

A Study of a Family with Dominantly Inherited Congenital (ant egg) Cataract Including a Histological Examination of the Formed Elements

BY

STEEN HOLST NISSEN and HENRIK DAA SCHRODER

A family with ant egg cataract in three generations is described The cataract is congenital probably of autosomal dominant inheritance Light microscopy of the ant eggs showed that they are made up of a peripheral zone of lens material and a large almost homogenous centre Element analysis by X ray spectrophotometry showed a high content of calcium and phosphorus in the centre The cataract has been easy to operate on and the postoperative visual results have been good

Key words ant egg cataract – zonular cataract – element analysis – X ray spectralanalysis – heredity

Ant egg cataract is a zonular cataract made up of ant egg like ovoid bodies arranged in a diffuse greyish opaque zone The ant egg cataract has been previously described by Axenfeld (1900) Stock (1902) Jaeger (1964) and by Riise who in 1967 reported several cases in two generations of one family Since then we have examined and treated several members of the third generation of this family The present publication extends the family tree thus contributing to our knowledge about the heredity and at the same time includes a histological study of the ant egg

Received June 2 1978

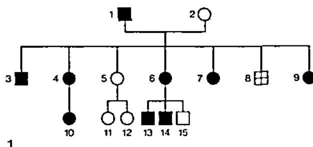


Fig 1
Pedigree Black figures patients with ant egg cataract Open figures family members without ant egg cataract Case 8 not examined

Observations

The cases 1-9 (Fig 1) were described by Riise (1967) On examination of the heredity we found as illustrated by the pedigree in three consecutive genera tions that the disease was represented in each generation and in nine out of 15 members Four of these are men One member (case 8) was not examined (died at the age of 15 months) Thus the heredity is seen to be autosomal dominant

Case Histories

Case 10 (150471) A 4 year old girl with failing vision for one year Visual acuity right eye 0 05 left eye 0 1 Dense ant egg cataract was seen in both lenses After aspiration of the cataract in the right eye, and three months later in the left visual acuity was 0 67 in both eyes with correction for aphakia on examination by the patient s own eye specialist

Case 11 (120971) A 6 year old girl with visual acuity 1 0 in both eyes without cor rection Clear lenses Normal ophthalmoscopy

Case 12 (210973) A 4 year old girl with visual acuity 0 67 in both eyes without cor rection Clear lenses Normal ophthalmoscopy

Case 13 (110171) A 6 year old boy with reduced vision At the age of three years visual acuity was 0 25 (E chart) in both eyes Examination showed typical ant egg cataract in both lenses After aspiration of the cataract in the right eye and eight months later in the left eye visual acuity was 0 67 in the right eye and 0 5 in the left eye with correction for aphakia Slit lamp examination showed in both eyes scattered ant eggs on the iris In the left eye a fairly dense secondary cataract was seen Normal ophthalmoscopy



Fi 2
An erer cataract. Case 14

Case 14 ...1973. A 4 year-old boy with bilateral cataract and an in ermi ent con vergent squint. Sept. 14 visual acuity 0... (E-chart) in both eyes. Aspir on of cataract in the right eye was performed and three months later in the left (Fig. 2). Jan. 1975 visual acuity was 0.6 in the right eye and 0.5 in the left with correction for aphakia. Slit lamp examination showed secondary cataract and a couple of ant-eggs on the iris. The aspirated material from the left lens was studied by light microscopy and element analysis.

Case 15 08.1574. Exam nation d fficult due to lack of coopera ion. No complaints or signs of reduced vision. Clear lenses and a normal fundus were observed on examination with an ophthalmoscope.

Method

The aspirated material from the cataract was fixed in cold 3% glutaraldehyde with 0.1 M phosphate buffer (pH 7.2). After three h fixation the tissue was postfixed for one h in 1% OsO_4 followed by dehydration in graded alcohol and embedded in Epon 812. Crude aspirate as well as dissected ant-eggs were embedded. Some tissue was demineralized for five days in 10% EDTA in 0.1 M tris buffer (pH 7) before postfixation and embedding.

For light microscopy 2 μm thick sections were stained with toluidine blue.

Some Epon blocks with an egg in the cut surface were coated with silver (evaporation technique) and analyzed for element content in a JEOL JAX 50A electron microprobe combined with an Ortec energy dispersive X ray spectrometer.

Light microsc The characteristic finding was that of globular structures with a diamete ... ut 500 μm (Figs. 3 and 4). They consisted of a major central greenish ... core which was stained weakly by toluidine blue and OsO_4. Much of ... core disintegrated during sectioning.

Surrounding the core lightly stained homogenous lens material was seen
At the border between the core and the surrounding area a zone containing densely stained material often organised in waved formed strands was seen (Fig 3) The border zone interdigitated with both the core and the surroundings In decalcified material the central core was preserved and was seen to consist of a heavily stainable material (Fig 4)

Analysis for elements The ant egg appeared as a bright area in the backscattered electron image (BEI) (Fig 5) Energy dispersive analyses from different points of the ant egg showed a content of calcium and phosphorus X ray scan for calcium and phosphorus across the structure demonstrated that the localization of these elements coincided with the bright area of the BEI (Fig 5)



Figs 3 and 4

Fig 3 Isolated ant egg Most of the calcified central zone is lost Rests of the unstainable material are seen at the open arrow At the thick arrow the wavy structure of the border zone is seen 3 μm toluidine blue × 380

Fig 4

Fig 4 Isolated decalcified ant eggs The organic material of the central zone can here be demonstrated The wavy border zone is seen at the thick arrow 3 μm toluidine blue × 380

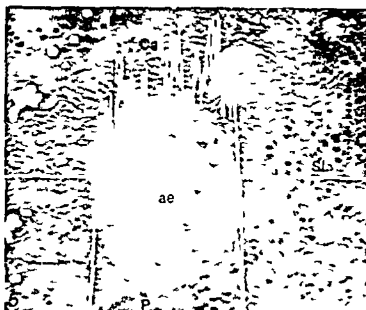


Fig 3
Backscattered electron image (BEI) of ant egg (ae) with superimposed plot at Ca and P concentrations obtained with line scanning BEI shows the size and shape of the ant-egg The scanning is performed along the line SL Base line is seen at the bottom of the picture ×350

Discussion

Ant egg cataract is a rare form of congenital cataract characterised by ovoid whitish structures arranged in a zonular area often most frequent in the anterior part of the lens (Fig 2) After surgical aspiration of the lens material the individual ant eggs were examined by light microscopy and by energy dispersive element analysis Electronmicroscopic observations of the ant eggs will be published in a subsequent paper

Ant egg cataract manifests itself clinically by considerable reduction of sight in early childhood The patients described in this article were operated on at the age of 3–4 years There have been no technical difficulties attached to the operation the ant eggs can be aspired at the time of operation without any difficulty As long as there is noe secondary cataract the vision was found to be good

On cutting ant eggs with a glass knife for semi thin sections the knife broke several times Thus the material was extremely hard

Stock (1902) studied by chemical and histochemical methods some crystal pearls of lens origin corresponding to the present ant eggs He observed that the pearls were insoluble in alkaline solutions in alcohol ether and chloro form but he found development of gas and dissolution of some components of the pearls during treatment with hydrochloric acid These results pointed to a content of calcium carbonate in the crystal pearls The histochemical staining methods for calcium phosphate (v Kossa and silver nitrate) employed by Stock did not demonstrate the presence of this compound

The multi elements analysis employed in the present study does not analyse for carbon moreover a scan for carbon would not distinguish between organic and inorganic carbon thus carbonate may be present in the ant egg However contrary to Stock s observation a content of phosphorus together with calcium was found by the energy dispersive analysis

Acknowledgments

The authors wish to express their gratitude to Mrs Ilona Bach Sørensen Mr Bjarne Krunderup Mr Albert Meyer and Miss Karin Wiedemann for their skilled technical assistance Special thanks are due to Dr Sidsel Fregerslev Institute of Geology Uni versity of Aarhus for the scanning electron microscopic examination of the tissue

References

Axenfeld T (1900) Praparate von Krystallperlen in der Linse *Ber dtsch ophthal Ges* 28 191–19?

Jaeger W (1964) Ameiseneierkatarakt *Ber dtsch ophthal Ges* 66 368–373

Riise R (196) Hereditary ant egg cataract *Acta ophthal (Kbh)* 43 341–346

Schrøder H D & Nissen S H Ant egg cataract An electron microscopic study (In preparation)

Stock W (190) Ueber Krystalperlen in Schichtstaren *Klin Mbl Augenheilk* 40 11–18

Author s address

Steen Holst Nissen M D
Department of Ophthalmology
Aarhus Kommunehospital
DK 8000 Aarhus C
Denmark

Department of Medical Biophysics
(Head Diego Carlström) and
Department of Ophthalmology Karlinska Sjukhuset
(Head Bjorn Tengroth)
Karolinska Institutet Stockholm

HUMAN TRAUMATIC CATARACT
A Quantitative Microradiographic and Electron Microscopic Study

BY

P P FAGERHOLM and B T PHILIPSON

Six cases representing different stages of cataract formation secondary to eye trauma were subjected to quantitative microradiographic or electron microscopic examination Anterior and posterior subcapsular cataracts were found to contain extensively swollen lens fibers in the subcapsular cortex Microradiographic measurements revealed a reduced concentration of dry mass in the subcapsular cortex around the whole circumference of the lens The inner cortex and the nucleus appeared normal both micro radiographically and electron microscopically

Two of the examined cases had an opaque cataract membrane and one had a Soemmerring s ring The opaque membranes consisted of irregular masse of degenerated lens fiber material as well as regenerated lens epi thelial cells A wide range of dry mass concentration was found in the opaque membrane that was studied microradiographically Alterations in morphology and dry mass concentration are more than sufficient to ex plain the development of opacification in traumatic cataract

Key words human lens – traumatic cataract – after cataract – ele tron microscopy – quantitative microradiography – light scattering

Trauma to the lens can lead to the formation of cataract Post trauma patholog may range from a local opacity at the site of the lens injury to an almost tota resorption of the lens leaving nothing but a membraneous after catarac (Davidson 1940a b Duke Elder 1972)

Received May 11 1978

The end result of the post traumatic events is dependent on various factors such as the age of the patient and the site and size of the injury. In general a minute injury will result in only a local opacification (Keeney 1971). A larger wound will cause formation of a posterior subcapsular cataract which often leads to a swollen and totally opaque lens. If the cataract is not aspirated at this stage, the major part of the lens fiber mass will be resorbed.

In an old lens the hard nucleus is generally resistant to a complete break down. The anterior lens capsule with its epithelium and the posterior capsule remain intact and may form a capsular bag enclosing cortical material and regenerated epithelium. Furthermore, regenerating lens epithelium can form an after cataract similar to that seen following extracapsular cataract surgery. The ultrastructure of two human traumatic cataracts secondary to penetrating injuries has earlier been studied by Rafferty et al. (1974).

In this study, different stages of cataract formation secondary to eye trauma were subjected to quantitative microradiographic or electron microscopic examination. The observations are compared to recent results obtained in experimentally induced traumatic cataract (Fagerholm & Philipson 1978a, b).

Materials and Methods

The lens specimens studied were selected in order to illustrate different stages of traumatic cataract. Within each stage there was a random selection of the cases analysed. (For detailed information see case reports.) All material was obtained from the Department of Ophthalmology, Karolinska Hospital, Stockholm in connection with eye surgery performed to restore vision.

The lens material was processed within 30 min following extraction after having been transported in Eagles MEM tissue culture medium.

All lens material was examined in a Haag Streit 800 slit lamp microscope (6–40 x) prior to extraction. Before processing the tissue, further examination was performed in a Wild 8M stereomicroscope.

Quantitative microradiography

The lens specimens were frozen in isopenthane precooled in liquid nitrogen to −140 C, freeze sectioned into 10–20 µm thick sections and freeze dried. Together with a reference system, the sections were mounted in close contact with a fine grained photographic emulsion and exposed to soft X rays generated at 3 kV. The microradiograms thus obtained were evaluated densitometrically in order to determine the dry weight of different regions of the lens. (For detailed information about the technique see Philipson 1969.)

Light and electron microscopy

The lens material was fixed in 1% glutaraldehyde in 0 1 M pho phate buff- (pH 7 4) for 30 min After postfixation in 1% osmium tetroxide and d hydra tion the specimen was embedded in Epon via propyleneoxide. Ultrathin sec tioning was performed on a LKB microtome The sections were stained in 3% uranylacetate and 6% lead citrate and examined in a Philips 301 electron microscope 1 μm thick secti ons were used in light microscopy examination.

Case Reports and Observations

Case 1

A 13 year old boy had at the age of 6 years suffered from perforation of the right eye by a thin needle A local opacification was seen at the site of the wound soon after trauma. A dense posterior subcapsular cataract then developed reducing visual acuity to less than 0 1 Cataract aspiration was performed after anterior capsulectomy

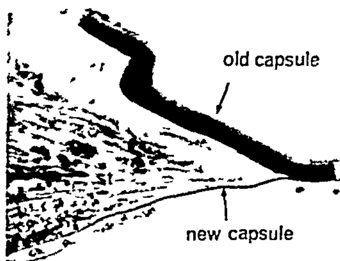


Fig 1

Light microgra h fr m a wo nd area obtained from a lens that seven years earlier suffered from a perforatin injury (Case 1) Epithelial cells have regenerated and formed a new thin capsule Ou side the regenerated epithelium a scar tissue is seen Toluidine blue 3 0



Fig 2

Electron micrograph from the central part of the scar tissue (Case 1) Around elongated cells layers of fibrous material is seen forming a large extracellular space × 2200

Light and electron microscopy of the excised part of the anterior capsule revealed a healed lens wound Scar tissue was seen between the rolled up capsular flaps (Fig 1) This tissue consisted mainly of elongated cells surrounded by abundant extracellular material (Fig 2) Underneath this scar tissue a single layer of regenerated epithelial cells was seen These cells had formed a new lens capsule

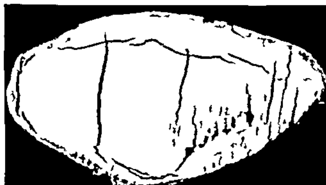


Fig 3

Microradiograph from a central section of a lens with traumatic cataract (Case 2) The lens had a thin zone of opacification in the subcapsular cortex A thin zone with low X ray absorption about 0 1 mm wide is seen in the entire subcapsular area × 10



Fig 4

Microradiograph from the same lens as in Fig 3 Detail from the posterior subcapsular cortex reveals a reduced dry mass content in the outer zone compared to the inner zone The formation of swollen cells is obvious Dry mass determinations are given as a mean value and a 99% confidence interval of 10 measurements at each point 1 0.09 ± 0.02 g/cm^3 2 0.35 ± 0.05 g/cm^3 ×25

Case 2

This 34 year old man's left eye had been perforated 22 years earlier by the edge of a steel band which caused a minor lens injury A slight posterior capsular opacification developed during the first year reducing visual acuity to 0.5 A later development of central retinal detachment reduced visual acuity to light perception Finally a therapy resistant glaucoma developed and the eye was enucleated In spite of these complications the lens appearance had not changed the slight posterior opacification not having progressed any further

Densitometrical evaluation of microradiograms obtained from lens sections of the region close to the optic axis revealed a low concentration of dry mass in the subcapsular cortex (Fig 3) This was found around the whole circumference of the lens Fig 4 shows the results of the densitometrical evaluation A very steep gradient in the concentration of dry mass was present at the borderline between this subcapsular zone and the inner cortex The inner cortex and the nucleus had normal concentrations of dry mass The subcapsular zone containing swollen lens fibers varied in thickness between 0.05 and 0.15 mm

Case 3

A 64 year old man had been hit in his left eye by the handle of a pair of garden scissors six months prior to cataract extraction. This blunt trauma caused backward lens subluxation and a herniation of the vitreous. A posterior subcapsular cataract developed the first months and remained relatively stationary until the time of surgery. Visual acuity was reduced to 0.1.

Extensively swollen lens fibers were most conspicuous in the anterior and posterior subcapsular cortex (Fig. 5). The lens epithelium appeared normal except in one region near the equator where a multilayered arrangement of the cells were seen (Fig. 6). No typical scar tissue could be found.

Case 4

A 30 year old man had 19 years earlier suffered from a perforation of the left eye caused by a thorn. A progressive traumatic cataract developed. At the time of surgery



Fig. 5
Electron micrograph from a human lens with traumatic cataract (Case 3). The epithelium (e) appears normal but lens fibers underneath are enlarged. × 4200



Fig 6

Light micrograph near the equator from the same lens as in Fig 5 (Case 3) A multi layered epithelium is seen Lens fibers are slightly swollen underneath Toluidine blue × 160

most lens material had been resorbed and the remaining tissue formed a dense opaque membrane reducing visual acuity to perception and localization of light The central part of this opaque after cataract was surgically extracted to restore vision

The biomicroscopic appearance of the after cataract and the electron microscopic findings were very similar to those of case 5 They will therefore be presented together with the observations made in case 5

Case 5

A 38 year old man had 20 years earlier suffered from a perforating nail injury to the right eye The nail had injured the lens in its anterior pole A traumatic cataract developed immediately The lens material eventually resorbed leaving only an after cataract consisting of a dense opaque membrane Visual acuity was reduced to perception and localization of light The central part of the membrane was surgically excised to restore vision

The opaque membranes excised in cases 4 and 5 had a similar appearance in the electron microscope The material was found to consist of two main components

a) degenerated lens fiber material (Fig 7) and

b) regenerated lens epithelial cells surrounded by abundant extracellular material (Fig 8) The degenerated fibers consisted of membrane enclosed spheres containing a substance of low stainability Membrane degradation products with myelin like appearance were seen Membrane remnants from adjacent

cells were also observed In the parts where regeneration had taken place the tissue consisted of elongated cells at different stages of degeneration surrounded by a large extracellular space This space was filled with layers of fibrillar material parallel to the cells

Case 6

A 94 year old woman was killed in a car accident The eyes were obtained about 4 h post mortem for the purpose of corneal grafting On examination one eye was discarded because of a corneal scar and an anterior synechia It was later established that the donor had suffered from a penetrating knife injury to the eye about 10 years earlier In place of the lens an after cataract was found consisting of a ring shaped semi transparent structure (a Soemmerring s ring) connected to a central opaque membrane

The microradiographic appearance of this after cataract was examined (Fig 9) The ring shaped structure was covered by a capsule The material en closed by the capsule consisted of lens epithelium and relatively intact lens cells Quantitative measurements of the concentration of dry mass revealed an even increase from the peripheral parts to the center of the Soemmerring s ring The highest values 0 44 g cm^{3} were found in the central region of the ring (Fig 9) The opaque membrane within the Soemmerring s ring consisted of irregular masses of lens material with a wide range of dry mass concentra tion (Fig 9)



Fig 7

Electron micrograph from a central part of an after cataract membrane (Case 5) Only scarce membranes are seen sometimes surrounding globuli with an amorphous less electron dense material The membranes are often discontinuous consisting mainly of junctions x 40 000

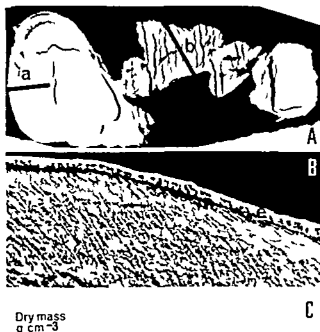


Fig 9

Micrograph from a section through a ring of Soemmerring (A) Densitometrical determinations have been performed along bar a and bar b (shown in C) × 11 B At the periphery an epithelial like structure (e) is seen inside a thin capsule × 125 C Diagram of the dry mass content along bar a and b in A The mean value and a 99 % confidence interval of 10 determinations at each point is given.

A further progression of the subcapsular cataract will cause a totally opaque lens with a swollen cortex Finally the lens will rupture In younger individuals the major part of the lens substance will be resorbed Generally the posterior lens capsule is left forming an after cataract (Cases 4 and 5) The posterior

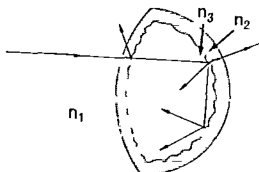


Fig 10
Schematic drawing of a lens with subcapsular reduction of dry mass concentration similar to that shown in Fig 3 Light scattering might occur at the interface between regions with different refractive indices The refractive indices given are calculated from determinations presented in Fig 4 Aqueous $n_1 \simeq 1.33$ hydrated cortex $n_2 \simeq 1.35$ and normal cortex $n_3 \cong 1.40$ One ray of light is marked and the main sources of light scattering are indicated The scattering of light is dependent on the angle of incidence Thus for these refractive indices the critical angle where total reflection occurs is 75 degrees At this angle total reflection occurs at the posterior interface between normal and hydrated cortex This posterior interface is wavy and irregular Thus the incident light frequently hits this interface at high angles causing an intense light scattering

Soemmerring s ring is often accompanied by a central opaque membrane which is irregular in its morphology and large local variations in dry mass probably intense light scattering This central membrane probably represents an after cataract membrane similar to those in Cases 4 and 5

A sharp interface was seen in Case 2 between areas with normal and reduced dry mass The interface found at the borderlines between transparent and opaque cortex corresponds to a jump in refractive index from about 1 35 to about 1 40 This change in refractive properties is sufficiently large to cause an intensive scattering of light especially if as in Case 2 the interface is irregular Light will then frequently hit the interface at angles close to the critical angle and consequently be totally reflected In Fig 10 a schematic example of this light scattering is shown Thus the physical background of the opacification with sharp jumps in the concentration of dry mass In the normal human lens local reductions of dry mass content are never found (Philipson & Fagerholm 1973) The morphological alterations discussed above along with the subsequent local reduction in the dry mass concentration are more than sufficient to explain the development of opacifications in traumatic cataract

The Eye Department of the Central Hospital of Middle Finland
(Head M Koskenoja)

LATE COMPLICATIONS OF 7–0 POLYGLYCOLIC (DEXON) SUTURES IN CATARACT SURGERY

BY

ANNELI KLEMETTI

The late complications of corneo scleral wound healing after the 15th postoperative day were recorded in 108 routine adult cataract operations using 7–0 polyglycolic acid (Dexon®) sutures and compared with 80 routine adult cataract operations using 9–0 monofilament nylon (9–10 Ethilon Nylon®) sutures
In 37 eyes of the 7–0 Dexon group filtrating blebs gaping of the corneo scleral wound anterior chamber collapse or decrease in the intraocular pressure were observed compared with one filtrating bleb in the 9–0 nylon group The majority of late complications occurred 22 to 42 days after surgery Ten filtrating blebs remained after a 5 months follow up period The most probable cause of the late complications in corneo scleral wound healing was discussed

Key words cataract surgery late complications – polyglycolic acid sutures Dexon®

The advantages of polyglycolic acid sutures (Dexon®) compared with many other suture materials are evident The good handling characteristics a diameter smaller than that of other absorbable sutures of equivalent tensile strength the non antigenic nature the minimal tissue reaction and the uniform and predictable absorption have made polyglycolic acid sutures useful in both intra and extraocular surgery (Furgiuele 1974 Sugar et al 1974 Sugar 1975 Williamson 1974 Helveston & Callahan 1976 Merrit et al 1974 White & Parks 1974)

Received August 18 1978

From experimental studies both after implantation in tissues other than the eye and after extraocular muscle surgery it appears that the tensile strength of polyglycolic acid sutures follows a uniform progressively decreasing pattern (Katz & Turner 1970 Craig et al 1975 Apt et al 1976)

In clinical series less attention has been paid to late complications which may be due to loss of tensile strength of the suture The purpose of this study was to determine the late complications (15 days or more after surgery) in cataract extractions using 7-0 polyglycolic acid sutures (7-0 Dexon*) compared with the use of 9 0 monofilament nylon sutures (9-0 Ethilon Nylon*) and to pay special attention to insufficiency in the healing wound (gaping and filtrations of the wound anterior chamber collapse and decreased intraocular pressure)

Dexon* is the registered trademark of Davis & Geck American Cyanamid Company Pearl River N Y 10965

Ethilon Nylon* is the registered trademark of Ethicon INC Sommerville N J USA

Material and Method

In 1976 all routine adult cataract operations in the Eye Department of the Central Hospital of Middle Finland were performed using 7-0 polyglycolic acid green sutures (7-0 Dexon*) with 3 8 Circle Lancet Needle LE 2 during the *first six months and 9-0 monofilament nylon sutures* (9 0 Ethilon Nylon*) with micro point cutting C needle during the remaining six months. Of the total 205 cataracts 15 9-0 Ethilon Nylon and 2 7-0 Dexon cataracts were omitted because of difference in the suturation technique In the remaining 188 cataract operations 7-0 Dexon sutures were used in 108 eyes and 9-0 Ethilon Nylon sutures in 80 eyes The operation technique is presented in Table 1 All patients were operated upon under local anaesthesia using 2% lidocain chloride with 1 200 000 adrenaline (epinephrine Lidocain Adrenalin*) for retrobulbar injection and to produce facial block Lidocain Adrenalin* is the registered trademark of Orion Helsinki 51 The operations were performed using Keeler s wide angle operating telescopic spectacles (magnification x 2 or x 4)

All 188 patients except one in the 9-0 Ethilon Nylon group had limbus based conjunctival flaps dissected followed by limboscleral incision with standard razon blade fragment (Keeler)

The wound was widened with corneal scissors to 160 -180 The number of 7-0 Dexon and 9-0 Ethilon Nylon sutures used in the corneo scleral wound were 6 to 10 placed interrupted

Table I

Operation techniques of 108 adult cataracts using 7–0 Dexon sutures and of 80 adult cataract using 9–0 Ethilon Nylon sutures in the closure of the corneo scleral wound

		7–0 Dexon (108 eyes)	9–0 Ethilon Nylon (80 eyes)
Limbus based conjunctival flap		108	79
Corneo scleral incision		108	79
corneal incision			1
Basal iridectomy		51	41
peripheral iridectomy		31	10
meridional iridotomy		26	29
Number of sutures	6	4	–
(corneo scleral wound)	7	72	38
	8	24	19
	9	6	16
	10	2	7
Alpha chymotrypsin			
used 0.1–1.0 ml		44	20
not used		64	60
Intracapsular extraction		103	74
Extracapsular extraction (planned)		5	3
Extracapsular extraction (not planned)		–	3
Extraction with cryophobe		103	76
Extraction with erisiphake		–	1
Extracapsular technique		5	3
Vitrectomy anterior		5	4
At end of operation			
Anterior chamber deepened with balanced salt solution		80	50
Anterior chamber deep without balanced salt solution		27	24
Anterior chamber flat		1	–
Closure of conjunctival flap continued 6–0 plain catgut		108	79

The depth of placement of the sutures was approximately ² ₃ of the corneoscleral tissue. Of the corneo scleral sutures usually three were preplaced prior to lens extraction.

The 7–0 Dexon sutures were tied with a double loop knot followed by two square knots. The 9 0 Ethilon Nylon sutures were tied with a treble loop knot followed by two square knots. Beginning with the first postoperative day all patients received 0.1 % dexamethasone drops four times daily and 1 % atropine sulphate twice daily during the hospital stay (usually 6 to 8 days).

The intraocular pressure was measured as a standard procedure on the 2nd, 4th and 6th day after surgery. Acetazolamide was used systemically when the intraocular pressure exceeded 22 mmHg. The first examination after discharge from hospital was performed 15 to 30 days postoperatively and the following examinations thereafter at 1, 2 and 3 to 6 months intervals counting from the operation. The whole material consisted of 66 males and 10? females in the 7–0 Dexon and the 9 0 Ethilon Nylon group together, ranging in age from 22 to 92 years. No difference in the ages of the 7–0 Dexon and 9 0 Ethilon Nylon groups could be observed (Table II).

There were seven patients with both eyes operated using 7 0 Dexon sutures and three patients with both eyes operated using 9 0 Ethilon Nylon sutures. Seven patients had one eye operated with 7 0 Dexon sutures and the other eye with 9 0 Ethilon Nylon sutures. The complications observed were verified by slit lamp microscopy. Only those cases in which the complications appeared

Table II

The age distribution of the patients in the 7 0 Dexon and 9 0 Ethilon Nylon groups

Age	7 0 Dexon (108 eyes)	9 0 Ethilon Nylon (30 eyes)
20 29 years	1	
30 39	–	
40 49	5	
50 59	21	4
60 69	33	[illegible]
70 79	34	5
80 89	13	14
90 – 99	1	1

Table III

The late complications observed in the 7-0 Dexon group and the 9-10 Ethilon Nylon group

	7-0 Dexon (108 eyes)	9-10 Ethilon Nylon (80 eyes)
Filtrating bleb and hypotony	17	1
Hypotony ≤ 6 mmHg	9	–
Suspected gaping of the corneo scleral wound and moderate hypotony 7-10 mmHg	8	–
Uveal prolapse	1	–
Choroidal detachment and hypotony	1	–
Intravitreal haemorrhage and hypotony	1	–
	37 (34.3 %)	1 (1.3 %)

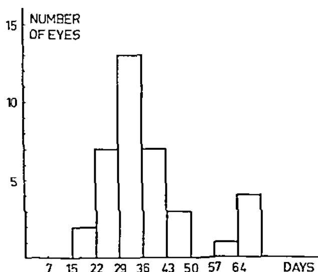


Fig. 1

The time of appearance of recorded late complications in the -0 Dexon group

15 days or more after surgery were included in the group of late complications None of these eyes showed any sign of wound insufficiency (filtering bleb gaping of the wound anterior chamber collapse or decrease in intraocular pressure) at the time of discharge from hospital or at examinations before the 15th postoperative day

Results

Table III summarizes the late complications observed after the 15th postoperative day in both the 7-0 Dexon and the 9 0 Ethilon Nylon group In the 7-0 Dexon series 5? eyes with late complications were registered and in the 9-0 Ethilon Nylon series complications were seen in only one eye The time of appearance of recorded complications is summarized in Fig 1 The earliest complications in the 7-0 Dexon group (2 cases) were detected 21 days postoperatively The majority of complications relating to the wound were however recorded between the 2?th and 4?th days after cataract extraction (? eyes) The only filtrating bleb in the 9 0 Ethilon Nylon group was not recorded until the follow up examination nine months after operation At the examinations one and two months postoperatively no filtration was observed

The follow up of the whole material is presented in Table IV

Follow up became more difficult because of the advanced age and poor health of many of the patients Seven patients died during the follow up period

Table IV
The follow up period of the whole material

Months	7-0 Dexon whole series (10? eyes)	9 10 Ethilon Nylon whole series (?0 eyes)
1 ?	14	11
3 - 4	?0	5
5 - 6	?1	15
7 - 8	21	12
9 - 10	?	5
11 - 1?	13	7
13 -	8	?1

Of the patients in the 7–0 Dexon group ,0 % have been followed up for five months or more and for the 9–0 Ethilon Nylon group the follow up per centage is 75

Ten of the 17 filtering blebs with 7–0 Dexon cataract included in the group of late complications still had the filtering bleb after a five to 11 months observation period In four cases the filtering bleb disappeared spontaneously and in three cases the follow up period was less than five months Reoperation has been performed in one eye of the 7–0 Dexon group (a transconjunctival cryoapplication)

No differences between the 7–0 Dexon and 9–0 Ethilon Nylon groups were observed with regard to the increased intraocular pressure on the 2nd and 6th day after operation use of alpha chymotrypsin or number of sutures in the corneo-scleral wound No statistical evaluations of these results were per formed because of the small number of cases in the groups studied

Discussion

The filtering blebs reported by Sugar (1975) in 11 cases of 156 cataracts with 6–0 Dexon sutures and in four cases of 70 cataracts with 7–0 Dexon sutures followed for six weeks to 1½ years were more frequent in the earlier perfor med operations Those cases were attributed to the disproportionately large needle size compared with the suture diameter Most of the blebs were sup posed to be due to variations in the depth of placement and too tight suturing

In these series the manufacturer s directions concerning the suturation tech nique were followed exactly and the needle size (3/8 circle Lancet Needle LE 2) was adequately proportioned to the 7–0 Dexon suture

None of the eyes in which late complications of the 7–0 Dexon group were recorded showed any sign of insufficiency of the wound at the time of dis charge from hospital Also the comparison of the late complications of the 7–0 Dexon group with those of the 9–0 Ethilon Nylon group (37 cases and 1 case respectively) excludes the probability of failure in wound closure

Very interesting is the time of occurrence of the late complications The earliest complications were recorded on the 21st postoperative day and in most cases the complications were registered 22 to 42 days after surgery

Experimentally Katz & Turner (1970) have shown that the *in vivo* tensile strengths of 4–0 3–0 2–0 0 and 1 size of Dexon sutures implanted sub cutaneously in rabbits had decreased 50 % at 11 days and about 80 % at 15 days The studies with rats by Craig et al (1975) indicate that both 2–0 and 4–0 size Dexon sutures retain the high original straight pull strength at seven

days postoperatively but then undergo a sharp decline in strength At 28 days after surgery there is no measurable breaking strength left The tensile strength of 5–0 Dexon sutures after extraocular muscle surgery in rabbits gives a similar result (Apt et al 1976)

The clinical manifestations of corneo scleral wound insufficiency in these series correspond to the experimental observations The results speak for the presumption that 7–0 Dexon sutures lose their tensile strength too rapidly before healing of the corneo scleral wound is completed Another possibility could perhaps be the suturation technique (too tight suturing) Without histological examination the possibility of necrosis in the suture canal as the primary cause cannot be excluded However in the light of these results 7–0 Dexon sutures cannot be recommended in cataract operations in which the long term retention of suture tensile strength is essential for wound healing

References

Apt L Gaffney W L & Dora A F (1976) Experimental suture studies in strabismus surgery *Albrecht v Graefes Arch klin exp Ophthal 201* 19–27

Craig P H Williams J A Davis K W Magoun A D, Levy A J Bogdansky S & Jones J P (1975) A biologic comparison of polyglactin 910 and polyglycolic acid synthetic absorbable sutures *Surg Gynec Obstet 141* 1–10

Furgiuele F P (1974) Ophthalmic use of a new synthetic suture (Dexon) A preliminary report *Ann Ophthal 6* 1219–1225

Helveston E M & Callahan M A (1976) Synthetic absorbable suture for strabismus surgery *Amer J Ophthal 82* 300–302

Katz A R & Turner R J (1970) Evaluation of tensile and absorption properties of polyglycolic acid sutures *Surg Gynec Obstet 131* 701–716

Merritt J C Chapman L & Rabb M (1974) Polyglycolic acid suture in strabismus surgery *Arch Ophthal (Chicago) 91* 439–440

Sugar H S (1975) Further use of polyglycolic acid (Dexon) sutures in cataract and other intraocular surgery *Ann Ophthal 7* 125–129

Sugar H S Lorfel R & Summer D (1974) Polyglycolic acid (Dexon) sutures in cataract surgery *Amer J Ophthal 77* 178–180

White R H & Larks M M (1974) Polyglycolic acid sutures in ophthalmic surgery *Trans amer Acad Ophthal Otolaryng 78* 632–636

Williamson D E (1974) The use of polyclycolic acid sutures in outpatient cataract surgery *Ann Ophthal 6* 333–340

Author's address

Anneli Klemetti
The Eye Department of the Central Hospital
of Middle Finland
40620 Jyvaskyla 62
Finland

Norwegian Defence Research Establishment
Division for Toxicology (Head Frode Fonnum)
Kjeller Norway

CHANGES IN PUPILLARY DYNAMICS IN YOUNG MEN DURING PROLONGED SEVERE EXERCISE

BY

RAGNAR LUND KARLSEN and NILS SØLI

Infrared pupillography has been performed on 9 male cadets taking part in a combat course lasting for 5 days The combat course involved lack of sleep strong physical exercise and caloric deficit During the course pupillary size was determined after rapid adaptation to darkness and the rate of concentration and the rate of dilatation after stimulation of the opposite eye with light was determined Dilatation time increased by 50 % towards the end of the course and pupillary size before light stimulation was reduced by 14 % On day 3 and 5 but not on day 1 small amplitude pupillary oscillations were observed The speed of contraction was however unchanged

Key words pupillary dynamics multifactorial stress

Each year cadets from the Norwegian Academy of War participate in a combat course lasting for 5 days In a similar course (1976) Opstad et al (1978) found by clinical examination that pupillary dynamics might be affected towards the end of the course It has in addition been reported (Yoss et al 1970) that lack of sleep is accompanied by small amplitude pupillary oscillations and reduced pupillary size This study was therefore undertaken to see how the pupils were affected by a severe multifactorial stress

Received September 4 1978

Materials and Methods

The subjects of this investigation were 9 male cadets of the Norwegian Military Academy participating in a ranger training course from Monday 12th (Day 1) June 1978 at 8 a m till Friday 16th (Day 5) June 1978 at 6 p m The cadets were between 22 and 25 years old (mean 24 2 ± 0 4) and had studied at the Academy for about one year They were healthy and in excellent physical and mental condition Due to slight injuries during the course two cadets could not be examined on day 3 and one on day 5

During the course the cadets were exposed to a combined stress of physical strain sleep deprivation and caloric deficit Previous work has shown that the physical exercise of the cadets was equivalent to 8–10 000 kcal/day (Waldum & Huser 1974) The amount of sleep was estimated to be 1–2 h totally during the course This estimate fits well with continuous pulse registrations during previous courses (Waldum & Huser 1974)

The course took place at about 500 m altitude in a forest area in the eastern part of Norway The weather was mostly good and was sunny and warm during the days (20–25°C) and cool at night (4–8 C) On day 1 and day 5 the cadets were tested at 5 a m in the same room (without windows) at a military camp on day 1 after 7 h of regular sleep On day 3 (when the course had lasted for 48 h) they were tested under more improvised conditions in a forest cabin at 9 a m

Pupillographic studies A Grundig TV camera model FA 70 was connected to a Sony Video Tape recorder model AV 3670 CE and an ordinary Sony TV monitor This combination made it possible for the examinator to simultaneously watch the eye on the TV screen and record on the video tape The display of a digital watch was placed temporal to the eye for time determination and was recorded simultaneously This made it possible to determine pupillary size as a function of time with an accuracy of 0 1 sec

Light conditions The examination was performed in darkness and the eye was illuminated with infrared light Due to the military program of the course not more than 10 min was available for each examination not allowing complete dark adaptation to occur

Light stimulation The contralateral eye was stimulated with a flash of centered light from a 20 W light bulb placed 0 5 m from the eye Light stimulation lasted for 1 ± 0 2 sec and was repeated three times with 1 min interval The switch of the light bulb was connected to the TV camera giving a signal to the tape recorder when the light was on

Table I

Effects of combined severe stress on pupillary dynamics studied by infrared pupillography on male cadets

	Pupillary size before light A_1 mm²	Pupillary size after light A mm	$A_1 - A$ mm²	Half time of contraction in sec	Half time of dilatation in sec	Number of cadets
Day 1	33 8 ± 2 8	15 6 ± 2 8	18 3 ± 1 7	0 61 ± 0 02	1 13 ± 0 17	9
Day 3	26 6 ± 3 2*	14 9 ± 3 1*	11 7 ± 1 6	0 57 ± 0 03	1 41 ± 0 18	7
Day 5	28 9 ± 2 8*	13 0 ± 1 4	15 9 ± 1 8	0 58 ± 0 03	1 09 ± 0 09**	8

The values represent mean ± sem The Wilcoxon signed rank distribution was used for statistics
Date are significantly different from Day 1 $*P < 0\ 01$ $**P < 0\ 05$

Treatment of data The results obtained from three successive light stimulations each day were pooled Pupillary diameter was measured but the results were expressed as pupillary area in sq mm Reduction in pupillary size after light stimulation was estimated The time for half maximal contraction and dilatation of the pupil was determined and was used as a parameter for the speed of contraction and dilatation The Wilcoxon signed rank test was used for statistical significance

Results

The most prominent observation in this study was that the half time of dilatation increased by 50 % on day 5 compared to day 1 The half time of contraction was however unaffected During the combat course a small reduction

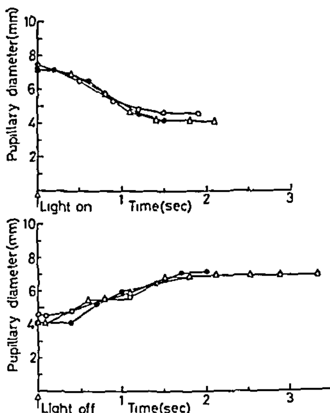


Fig 1

A typical example (cadet 8) of the pupillary response to the three successive light stimulations (top concentration bottom dilatation) before the course started (Day 1)

in pupillary size (A_1) was observed The effect was more pronounced on day 3 than on day 5 Pupillary size after light stimulation was unaffected during the course (A) The reduction in pupillary size after light stimulation ($A_1 - A$) was most pronounced on day 1 but the differences were only significant be tween day 1 and day 3 and between day 3 and day 5 (Table I)

Five out of 9 cadets had small amplitude pupillary oscillations with a fre quence of 0 2 sec on day 3 and day 5 which was never observed on day 1 A typical example of the pupillary response to light stimulation is given in Fig 1

Discussion

This study was done during a ranger combat course under field conditions necessitating the use of relatively simple instruments for the pupillographic studies The military program of the combat course was on a very tight time schedule not allowing enough time for complete dark adaptation which would have been desirable The test conditions on day 1 and 5 were very similar so that the differences observed between these two days are most probably due to severe combined stress the cadets had received In contrast to this on day 3 the cadets were rapidly transferred from bright sunshine to the darkness of the examination room The changes observed on day 3 compared to day 1 and day 5 may therefore in part be due to the different conditions under which the examination took place

On day 5 the pupillary size was reduced by 14 % compared to day 1 in agreement with previous work demonstrating that lack of sleep is accompanied by reduced pupillary size (Yoss et al 1970) On day 3 however pupillary size was reduced even more (21 %) and this may in part be due to in complete dark adaptation The following discussion will therefore be restricted to the differences observed between day 1 and day 5 The small amplitude pupillary oscillations observed on day 5 but not on day 1 have also previously been de scribed following lack of sleep (Yoss et al 1970) and may be interpreted as an imbalance between the sphincter and dilator tonus of the pupil

The most prominent observation in this study was however that the pupil dilated much more slowly towards the end of the course whereas the speed of contraction was unaffected This observation suggests that the dilator function of the pupil is more sensitive to prolonged combined stress than the sphincter function and this may in part be the explanation for the pupillary oscillations

The test subjects were exposed to a severe combined stress of physical strain sleep deprivation and caloric deficit It is therefore from this study impossible

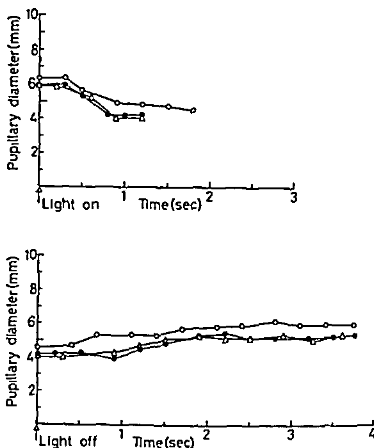


Fig ?

Pupillary response to the three successive light stimulations (cadet 8) at the end of the course (Day 5) top concentration bottom dilatation Note the pupillary oscillations at the end of the dilatation period and the slower dilatation of the pupil

to say which of these factors were responsible for the differences observed As for the underlying mechanisms the interpretations would be purely speculative and are therefore omitted

Acknowledgments

The authores are indebted to Tor Hoimyr and Eivind Stromman for valuable technical advice

This study was promoted by the Stress Research Group of Joint Norwegian Medical Military Service

References

Opstad P K Ekanger R., Nummestad M & Raabe N (1978) Performance mood and clinical symptoms in men exposed to prolonged severe physical work and sleep deprivation *Aviation Space and Environmental Medicine* (in press)

Waldum H L & Huser P O (1974) Stress reactions during strenuous military training (Norwegian) *Sanitetsnytt I* 1–18

Yoss R E Moyer N J Carter E T & Evans W E (1970) Commercial airline pilot and his ability to remain alert *Aerospace Medicine 41* 1339–1346

Authors addresses

Norwegian Defence Research Establishment
Division for Toxicology
P O Box 25 N 2007
Kjeller
Norway

Department of Ophthalmology (Head I Takats)
University of Pecs Hungary

RELATIONSHIP BETWEEN BLOOD FLOW VELOCITY IN THE CHOROID AND INTRAOCULAR PRESSURE IN RABBITS*

BY

I TAKATS and F LEISZTER

By using thermistors for calorimetry to monitor blood flow velocity in the choroid of the anaesthetized rabbit it was found that an elevation of intraocular pressure reduced the choroidal blood flow The relationship between the ratio of perfusion pressure to intraocular tension (x) and the reciprocal value of the percentage of blood flow velocity (l/y) showed a curve convex towards the pressure axis On a log lin scale the graph was linear A significant linear correlation with a coefficient of 0 965 was obtained The regression equation was found to be log l/y = 0 48x − 1 98 ± 0 29 From the present experiments it is concluded that the vascular bed of the choroid in the rabbit is a passive one with no sign of autoregulation The observations are discussed from the point of view of blood supply and possible damage to the optic disc

Key words choroid – vascular bed – blood flow velocity – ocular pressure – *perfusion pressure – passive circulation – autoregulation* – calorimetry – rabbit

It is common knowledge that in the dynamics of fluid circulation – as in electrodynamics – three factors are active 1) the volume of the circulating fluid (intensity) 2) differential pressure (voltage) and 3) resistance of flow (resistance) The relationship of the three factors is $F = \frac{\Delta P}{R}$ The application of this

* Supported by the Scientific Research Council Ministry of Health Hungary Grant Number 3 ?8 0301 04 ?/T

Received June 13 1978

relationship (Hagen Poiseuille law applicable to Newton s fluid) to blood circulation in a closed system of pipes makes several considerations necessary Viz R is directly proportional to the viscosity and length of the pipe and inversely proportional to the product πr^4 The vascular wall is however elastic and the blood contains corpuscles etc Yet with certain reservations about the capillary system this law may be applied to the circulation of blood As compared with other parts of the body certain differences which concern the intraocular vascular system first of all that of the choroid should be emphasized The diameter of the capillaries of the choroid is three times that of the usual The pressure in the small arteries is relatively high (Duke Elder 1926 Bill 1963 Weigelin & Lobstein 1963 Takats 1970) Since the arterioles in the choroid are only short stumps the pressure in the capillaries is remarkably high It follows from what has been said that the blood supply to the eye and particularly to the choroid is the highest in the body

A further characteristic is that the pressure exerted by the tissue on the vessels is the highest in the eye they are under a pressure of 15–20 mmHg From this it follows that the pressure in the venules has to be at least as much for the blood to be able to leave the eye At the same time there is a great fall in pressure in the veins passing through the sclera Extraocular venous pressure is 4–5 mmHg lower than intraocular pressure (waterfall phenomenon)

In the past decades interest has been focussed on the relationship between intraocular pressure i e perfusion pressure and choroidal circulation as it has been proved that the prelaminar region of the optic nerve head is supplied mainly by centripetal branches from the adjacent peripapillary choroid (Hayreh 1972) It is known from investigations by Goldmann & Blok (1971) that the choroidal vessels are particularly sensitive to a rise in intraocular pressure The latter authors applied mild pressure to the eyes of normal persons in response to which the blind spot was found to enlarge

A knowledge of the relationship between intraocular pressure i e a fall in perfusion pressure and choroidal blood flow is of great importance for the damage to the optic nerve in glaucoma It is questionable if the vascular system of the choroid is capable of some autoregulation to compensate for the rise in intraocular pressure

The aim of the investigations to be described was to determine the relationship between perfusion pressure and blood flow through the choroid in the case of pathological values of intraocular pressure Similar examinations which however yielded different results were carried out by Niesel (1962) Friedman (1970) Alm & Bill (1973) and Armaly & Araki (1975)

Materials and Methods

A total of 26 New Zealand albino rabbits were used The animals were anaesthetized with urethane (1 0–1 5 g/b wt) injected into the marginal ear vein To ensure good respiration a polyethylene tube was inserted into the trachea Blood flow velocity was determined calorimetrically with a heated and a non-heated thermistor as described previously (Takats 1973) The thermistors were each introduced into a glass capillary one of whose ends had previously been sealed up After incising the conjunctiva the glass capillaries were fixed with 6/0 silk sutures to the temporal part of the sclera immediately behind the equator The two thermistors were 5–10 mm apart In order to isolate them from their surroundings the thermistors were covered with moistened cotton wool The palpebral fissure was closed with sutures The leads from the heating manganine wire and those from the thermistors were connected to a Wheatstone bridge as described by Seylaz (1965) The changes in intensity of the electric current proportional to the blood flow velocity were recorded on an X Y recorder (Videoton Hungary) The intraocular pressure was changed by means of a needle placed in the anterior chamber The needle was connected to a reservoir filled with mock aqueous humour and a pressure transducer A separate pressure transducer measured the mean arterial pressure through a cannula introduced into the left femoral artery the changes being recorded by a Kipp Zonen micrograph

Blood flow velocity being directly proportional to blood flow through the tissue (Seylaz) it seemed justifiable to regard a decrease in blood flow velocity as a decrease in the volume of blood flow According to the basic principle of the bridge blood flow velocity is directly proportional to the intensity of the electric current necessary for maintaining the difference in temperature between the two thermistors This intensity of current on the other hand is directly proportional to the changes of the curve recorded by the recorder (e g 25 mA = 10 mm 50 mA = 20 mm) The percentage changes in blood flow velocity were calculated from the changes of the curve recorded by the recorder the base line corresponding to an intraccular pressure of 20 mmHg which was taken as 0 per cent The intraocular pressure was increased above the level of the systolic pressure in the femoral artery by raising the reservoir as a result of which the choroidal circulation was stopped The resulting change of the curve was taken as 100 per cent The changes of the curve associated with the different values for intraocular pressure were expressed as percentages of the maximum deviation obtained in the manner described above After resetting intraocular pressure at 20 mmHg a 10–15 min period of rest was allowed and then the intraocular pressure was increased stepwise 10 mmHg at a time by raising the reservoir until the change in the rate of choroidal blood flow c

Results

The aim of the experiment was to determine the relationship between intra ocular pressure and choroidal blood flow Considering that there was a great difference in blood pressure between the animals the perfusion pressure (mean femoral artery pressure minus intraocular pressure) was taken as one of the factors of our calculations We had three values at our disposal intraocular pressure mean femoral artery pressure and the percentage of the slowing of choroidal blood flow The change in choroidal blood flow being a function of the intraocular pressure the data obtained were plotted on a graph whose abscissa represented the perfusion pressure in relation to the intraocular pres sure which enabled a better approximation to the two values

$$\frac{\text{mean femoral artery pressure} - \text{intraocular pressure}}{\text{intraocular pressure}}$$

On the ordinate were plotted the reciprocal values for the percentage of the slowing of choroidal blood flow The diagram obtained in this way gave a curve convex toward the pressure axis

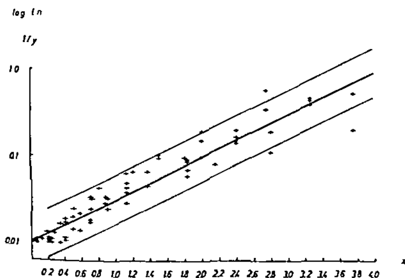


Fig 1
Reciprocal percentage of the slowing of choroidal blood flow (l/y) and perfusion pressure in relation to the intraocular pressure (x) on a log lin scale

In order to determine the exponent of the power function of the curve, a system of coordinates was used in which the logarithms of the above values were plotted on the ordinate

Seventy six reliable experimental values could be represented in the graph Using Gauss principle of the smallest squares a regression line was drawn to the points plotted in the logarithmic linear graph (Fig 1) The correlation coefficient of this line was significant at the level of $P < 0.001$ The relation represented by the above mentioned function may be regarded as linear ($r = 0.965$)

On the basis of our results the following interrelationship may be established between the ratio of perfusion pressure to intraocular pressure and the rate of choroidal blood flow $\log 1/y = 0.48x - 1.98 \pm 0.29$ (P = probability of 95%) where y = percentage of the slowing of choroidal blood flow x = ratio of perfusion pressure to intraocular pressure

Discussion

In the present decade the investigations into the relationship between intraocular pressure and choroidal blood flow have not led to unambiguous results. Friedman (1970) who injected a solution containing krypton 85 into one of the long posterior ciliary arteries of a cat drew the conclusion that the vascular system of the choroid behaved like a passive vascular bed up to 30 mmHg above that level of pressure an active regulatory mechanism was brought into action Weiter et al (1973) using radioactively labelled microspheres 50 μm in diameter found that choroidal blood flow in the cat was stable up to 30 mmHg and that it was only above that value that it began to decrease linearly On the basis of determining the amount of blood passing through one of the vortex veins Nakamura & Goulstine (1973) found that above the level of 20 mmHg of intraocular pressure the rate of blood flow decreased in rabbits From his examinations performed with a Doppler flow detector Tokoro (1972) reached the conclusion that below 25 mmHg blood flow velocity did not change above that value the relationship between ocular tension and the decrease in blood flow velocity was linear However what he examined was the ciliary body and not the choroid

In our own calorimetric examinations (Takats 1976) we also found that *on increasing the intraocular pressure from 15 mmHg to 25 mmHg in rab*bits choroidal blood flow began to slow The investigation carried out by Alm & Bill (1973) with the help of radioactivity labelled microspheres 15 μm in

diameter deserves attention On increasing the intraocular pressure by 20 5 mmHg in monkeys the quantitative decrease in choroidal blood flow seemed linear Using the heated thermocouple principle in cats and monkeys Armaly & Araki (1975) found that the choroid behaved like a passive vascular bed when the intraocular pressure was raised The latter authors repeated Friedman s ex periments and observed that the retrograde injection into the ciliary artery of a solution containing krypton 85 distributed the choroidal blood flow and led to errenoeus results

The present investigations also seemed to confirm the observation that in rabbits choroidal blood flow decreases linearly with decreasing perfusion pres sure no autoregulative mechanism manifested itself

We are aware that the results obtained in rabbis should be viewed with re servation The Seylaz method determines blood flow velocity and not volume of blood Although as used under the conditions of the present investigation the method may be regarded as semiquantitative Before our investigation Bill (1967) and Armaly & Araki (1975) who used a method which differed from ours but was still calorimetric drew a similar conclusion as to the relationship between intraocular pressure and choroidal blood flow It should also be taken into account that all these experiments those of others as well as ours were of an acute nature The question may be raised whether a prolonged increase of 5–10 mmHg in the human eye does not call some regulatory mechanism in action clinical observations have not decided it unambiguously According to several clinicians an intraocular pressure above the level of 22 mmHg con tinuing over a period of years results in damage to the optic disc According to others in the case of the so called ocular hypertension even 30 mmHg will not give rise to damage to the optic disc or to visual field defects (Koller & Becker 1977)

It may be that for moderate variations in intraocular pressure there is a re gulatory mechanism which varies with the individual in range and vulner ability

Within the limitations of the technique used our investigations permit the conclusion that in response to an acute increase in intraocular pressure in the rabbit the vessels of the choroid show no sign of autoregulation of blood flow

Only a non invasive quantitative method applicable to man will enable final conclusions to be drawn In this respect the method of Cristini et al (1975) may be regarded as an encouraging step There is hope that by further increasing the sensitivity of the method the question of whether or not there is a regula tory mechanism in the choroid for moderate increases in intraocular pressure may be decided

References

Alm A & Bill A (1973) Ocular and optic nerve blood flow at normal and increased intraocular pressures in monkeys (Macaca irus) A study with radioactivity labelled microspheres including flow determinations in brain and some other tissues Exp Eye Res 15 15–29

Armaly M F & Araki M (1975) Effect of ocular pressure on choroidal circulation in the cat and Rhesus monkey Invest Ophthal 14 584–591

Bill A (1962) Autonomic nervous control of uveal blood flow Acta physiol scand 56 70–81

Bill A (1963) Blood pressure in the ciliary arteries of rabbits Exp Eye Res 2 20–4

Cristini G Medori R Carbini C C & Giovanni A (1975) A new method for determining the blood quantity in the eye in a unit of time Albrecht v Graefes Arch klin exp Ophthal 194 1–11

Duke Elder W S (1926) The ocular circulation Its normal pressure relationship and their physiological significance Brit J Ophthal 10 513–572

Friedman E (1970) Choroidal blood flow Pressure flow relationship Arch Ophthal (Chicago) 83 95–99

Goldmann H & Blok P (1971) On a possibility to connect quantitatively ocular hypertension and damage to the optic nerve Albrecht v Graefes Arch klin exp Ophthal 183 231–243

Hayreh S S (1972) Optic disc changes in glaucoma Brit J Ophthal 56 175–185

Koller A F & Becker B (197) Ocular hypertension vs open angle glaucoma A different view Arch Ophthal (Chicago) 95 586–587

Nakamura J & Coulstine D (1973) The effect of intraocular pressure on the vortex vein blood flow Exp Eye Res 15 461–466

Niesel P (1962) Messungen von experimentell erzeugten Änderungen der Aderhautdurchblutung bei Kaninchen Karger Basel/New York

Seylaz J (1965) Développement d une méthode de mesure instantanée localisée et semi quantitative du débit sanguin in vivo Biophysik 2 330–339

Takáts I (1970) Data on the relationship between the blood pressures of the ciliary and femoral arteries (Hung) S emes et 107 248–252

Takats I (1973) Einfluss von Succinylcholin auf den intraokularen Druck im Tierexperiment Acta chir Acad Sci Hung 15 383–387

Takats I (1970) Investigations into the relationship of intraocular tension and blood circulation of the uvea in rabbits (Hung) S emes et 113 2–6

Tokoro T (1972) Relationship between the blood flow velocity in the ciliary body and the intraocular pressure Invest Ophthal 11 945–954

Weigelin E & Lobstein A (1963) Ophthalmodynamometry Hafner New York

Weiter J J Schachar R A & Ernest J T (1973) Control of blood flow I Intraocular pressure Invest Ophthal 12 327–331

Authors address
Department of Ophthalmology
University of Pécs
Hungary

Department of Ophthalmology (Head Jan Ytteborg)
Ulleval Hospital University of Oslo

PILOCARPINE MEDICATION IN OPEN ANGLE GLAUCOMA

A Study Using Pilocarpine Eyedrops and an Ocular Therapeutic System

BY

O BRINCHMANN HANSEN and N ANMARKRUD

Two modes of topical pilocarpine therapy were applied to sixteen patients with open angle glaucoma A pilocarpine drop medication (2% solution four times daily) was compared to a constant and continuous supply of pilocarpine by a therapeutic delivery system (Ocusert P 40) The pressure reducing efficiency was studied in the morning and in the following four hour period of an ordinary drop medication regimen
The results indicate about equal pressure reducing properties in both high and low pre treatment pressure values However compared to pilo carpine drops we found a statistically significant lower pressure in the morning after the Ocusert unit especially in glaucomatous eyes with high pre treatment pressure

Key words open angle glaucoma – pilocarpine – Ocusert – intraocular pressure

For a century (from 1877) pilocarpine has been administered by eye drop solu tions to patients suffering from different kinds of glaucomas In clinical usage a topical instillation is applied to the glaucomatous eye in a pulsed manner of medication usually four times daily during the waking hours of the patient This mode of ocular self medication has proved itself very useful in reducing the intraocular pressure Failure to get an expected pressure lowering response from drop medication could either be the result of inefficiency of the pre scribed therapy or it could be the result of poor patient compliance (Armaly & Rao 1973)

Received September 27 1978

An ocular therapeutic system (Ocusert P 40) worn in either the upper or lower cul de sac will supply a glaucomatous eye with pilocarpine molecules at a constant rate of 40 μg/h This continuous release of drug has been shown to reduce the intraocular pressure substantially with less total amount of pilocarpine applied to the eye when compared with the pulsed dose medication (Armaly & Rao 1973) In the last few years papers have been published comparing drops and membrane units with regard to dose effect relationship both in normal eyes (Place et al 1975) and in hypertensive/glaucomatous eyes (Armaly & Rao 1973 Macoul & Pavan Langston 1975 Quigley et al 1975) These works show that the membrane controlled delivery system and the pilocarpine eyedrops achieved hypotensive results both in normal and open angled glaucomatous patients Quigley et al (1975) found that a 40 μg/h unit controlled eyes requiring 2% and 4% pilocarpine solutions four times daily Other writers have been dealing with the Ocusert unit in other respects patient compliance (Friederich 1974) side effects (less miosis and less myopia) (Brown et al 1976) and 24 h studies of effects of constant drug delivery to the glaucomatous eye (Armaly & Rao 1973)

The purpose of this study was to compare the two modes of topical pilocarpine medication in open angle glaucomas with regard to the pressure reducing efficiency We wanted to study the effect on the intraocular pressure in the morning at a time just before a glaucomatous eye would receive a pilocarpine drop We also wished to compare the effect on IOP during the following 4 h

Material and Methods

This study included sixteen patients with open angle glaucoma all of them had previously been treated with pilocarpine in drop solution and all were responsive to this drug They were free from external eye diseases and none had undergone surgical eye intervention All of them had earlier been diagnosed as having glaucoma The patients were included based on following criteria intraocular pressure equal to or greater than 22 mmHg measured by Goldmann applanation in the sitting position and glaucomatous cupping of the optic disc with or without nerve fiber damage A Haag Streit lamp and mydriatics were used to reveal pseudoexfoliation of the lens capsule All of the patients were examined just prior to the study

Pseudoexfoliation of the lens capsule was found in eight patients There were 10 men and six women and the average age was 66 years (53–79) Number of eyes with glaucoma was 26 As a pre study regimen five eyes used epinephrine (four patients) two eyes used acetazolamide (one patient) and five eyes used

epinephrine and acetazolamide (three patients) The patients who used epi nephrine and/or acetazolamide continued the same medication throughout the study

The mean pressure of three readings were used no measure varying more than 3 mmHg from the others The measurements were done by alternating between the patient s right and left eye

Each patient was subjected to two experimental treatment sessions Each session was preceded by a 44–48 h period during which time any pilocarpine medication was discontinued In the middle of this period the pressure was read at 9 a m in order to establish pre treatment pressure values In the one session the patients started a drop medication with one drop four times during one day (9 a m 1 p m 5 p m and 9 p m) The IOP was measured the next morning at 9 a m After this reading the patients were given by us one pilo carpine drop topically and the IOP was again measured at 11 a m and 1 p m In the other session on Ocusert unit was placed in either the upper or lower cul de sac at 9 a m and the IOP was measured the next morning at the

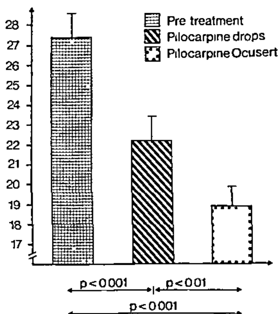


Fig 1
Mean ± SEM in 25 eyes at 9 a m Significant differences between treatments given by *P* values

same hour of the day The patients continued wearing the unit and the IOP was again measured at 11 a m and 1 p m The sequence of these sessions w... randomly assigned for each patient

Results

All sixteen patients completed the study They were well instructed how to replace the Ocusert unit in the lower cul de sac if the unit should fall out. We started out with 26 eyes five eyes lost the unit during the 24 h trial time The unit were at once replaced with the exception of one eye thus 25 eyes completed the study

The Student s t test (with $P < 0\ 05$) was used in the statistical calculations.

Table I

Mean IOP values during a four hour period in the two groups and in all patients after pilocarpine drops and Ocusert P 40 respectively

		Pilocarpine drops			Ocusert P 40		
		9 a m	11 a m	1 p m	9 a m	11 a m	1 p m
a)							
Group I	Mean	18 8	17.5	18.33	17 4	18 1	19.94
IOP < 26 mm	SEM	1 03	0 84	0 75	0 55	0 86	0 9
n = 12	Significance	3 57	2 92	2 61	1 97	3 01	3.39
		— n s —	— n s —		— n s —	— n s —	
		— n s —			— $P < 0\ 05$ —		
b)							
Group II	Mean	25 05	20 79	20 70	20 36	20 51	20 68
IOP ≥ 26 mm	SEM	1 64	1.25	0 89	1 68	1 4	1 49
n = 13	Significance	5 93	4.50	3 21	6 08	6 78	5 35
		– $P < 0\ 005$ –	– n s –		— n s —	— n s —	
		— $P < 0\ 005$ —			— n s —		
c)							
Group I and II	Mean	22 05	19 45	19 56	18 94	19 6	20.33
	SEM	1 15	0 75	0 6[illegible]	0 94	0 99	0 59
n = 2	Significance	5 79	3 75	3 12	4 74	4 9	4 46
		– $P < 0\ 005$ –	– n s –		— n s —	— n s —	
		— $P < 0\ 005$ —			— n s —		

The effect on applanation pressure level

Fig 1 gives the mean IOP reducing efficiency (measured at 9 a m) of the eyedrops and the unit in all 25 eyes Both modes of pilocarpine administration reduced the pre treatment pressure by a statistically significant amount ($P < 0.001$) The protection against pressure rise in the morning however was greater using the Ocusert unit during the sleeping hours than the carrying over effect of the drops This difference was statistically significant at a level $P < 0.01$

In Table I a b we did split the material in a high and a low pre treatment pressure group We chose IOP = 26 mmHg as a suitable level and we wanted to study the pressure reducing efficiency of the unit and the drops during a four hour period (9 a m – 1 p m) Figs 2 and 3 point out the advantage of using the constant drug delivery system especially in a group with high pre treatment intraocular pressure In the lower pressure group the carrying over effect of the drop medication seems to keep the IOP down to an acceptable level

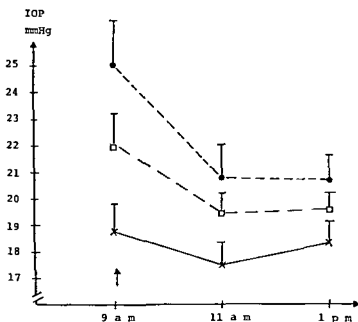


Fig 2

The effect on IOP during the four hour period after a single dose of pilocarpine drops (at 9 a m) (↑) (mean ± SEM)

×——× = group I ●– – – –● = group II and □– – –□ = both groups

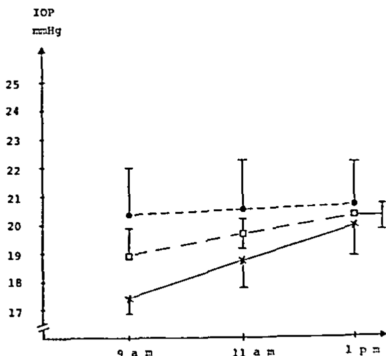


Fig 3
The effect on IOP during the four hour period during continuous use of the Ocusert unit
×——— = group I ●- - - - -● = group II and □ - -□ = both groups

After having received the morning pilocarpine drop both the Ocusert and the drop medications are about equal in their pressure lowering capabilities during the next four hour period

Comments

Studies dealing with diurnal variations of the intraocular pressure (in both normal and glaucomatous eyes) seem to agree upon the importance of controlling the IOP especially during the morning and early part of the waking hours (Henkind et al 1973 Kitazawa & Horie 1975 Worthen 1976) These workers used frequent measurement during 24 h periods and found peak diurnal intraocular pressures in most subjects in the morning and early daytime Our IOP readings were done in the morning at a time just before glaucomatous eyes would usually receive pilocarpine in drop medication This critical

morning period is probably the least protected against pressure rise of all periods during the waking hours of patients with open angle glaucomas

Davanger (1964) has pointed out by use of hydrodynamic laws that the effect of miotics (in normal and glaucoma simplex eyes) increases in a systematic manner with increasing pressure and Krill & Newell (1964) point out in a clinical trial that the higher the average pre treatment tension the greater is the reduction produced by any strength of pilocarpine

This study confirms the efficiency of both the unit and the drops in reducing the intraocular pressure in open angle glaucomas Both modes of topical pilocarpine administration seem to have about equal pressure lowering properties in both high and low pre treatment pressure groups

The study also shows that the carrying over efficiency of pilocarpine drop medication during the sleeping hours is considerably less than the efficiency of the ocular therapeutic system used here The degree of insufficiency in reducing the morning IOP prior to the first pilocarpine drop of the day seems to increase *with increasing pre treatment pressure level*

A membrane controlled delivery system seems to be a better topical pilocarpine regimen compared to drop solutions in the treatment of open angle glaucoma with high pre treatment intraocular pressure

References

Armaly M F & Rao K R (1973) The effect of pilocarpine Ocusert on ocular pressure In Leopold I H Ed *Symposium on Ocular Therapy 6* 80–94 C V Mosby St. Louis

Brown H S et al (1976) Visual effects of pilocarpine in glaucoma *Arch Ophthal (Chicago) 94* 1716–1719

Davanger M (1964) The pressure reducing effect of miotics in glaucoma simplex and in normal eyes elucidated by hydrodynamic calculations based on Poiseulle s law *Acta ophthal (Kbh) 42* 3– 81

Friederich R L (1974) The pilocarpine Ocusert A new drug delivery system *Ann Ophthal 6* 1279–1284

Henkind P et al (1973) The diurnal curve in man New observations *Invest Ophthal 12* 705–707

Kitazawa Y & Horie T (1975) Diurnal variation of intraocular pressure in primary open angle glaucoma *Amer J Ophthal 79* 557–566

Krill A E & Newell F W (1964) Effects of pilocarpine on ocular tension dynamics *Amer J Ophthal 57* 34–40

Macoul K L & Pavan Langston D (1975) Pilocarpine Ocusert system for sustained control of ocular hypertension *Arch Ophthal (Chicago) 93* 587–590

Place V A et al (1975) Comparative pharmacologic effects of pilocarpine administered to normal subjects by eyedrops or by ocular therapeutic systems *Amer J Ophthal 80* 706–712

Quigley H A et al (19,5) Pilocarpine Ocusert *Arch Ophthal (Chicago) 93* ,71–775
Worthen D M (19,6) Effect of pilocarpine drops on the diurnal intraocular pre sure variation in patients with glaucoma *Invest Ophthal 15* 784–78,

Author s address
Dr Olaf Brinchmann Hansen
The Eye Department
Ullevål Sykehus
Oslo 1
Norway

Department of Ophthalmology
(Heads P M Møller E Goldschmidt S Faurschou)
University Hospital Odense Denmark

CAPILLARY HAEMANGIOMA OF THE OPTIC DISC

A Case Report

BY

P GILBERT NIELSEN

A case of capillary haemangioma of the optic disc is reported The ophthalmoscopic and fluorescein angiographic characteristics of the different varieties of haemangioma of the optic nerve head are discussed and the problems of treatment by photocoagulation outlined

Key words haemangioma – optic disc – fluorescein angiography – photocoagulation

Angiomatosis retinae and von Hippel s disease are terms used synonymously to describe capillary angiomatous hamartomas of the retina and the optic nerve (Gass 1974) The development of an angioma in the optic disc or juxtapapillary retina is very uncommon This report documents with the help of fluorescein angiography a case of capillary haemangioma of the optic disc The various types of angiomas of the optic nerve head are tabulated and the problems of management outlined

Case Report

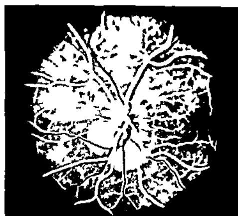
A 24 year old woman complained of blurred vision in the left eye some two months prior to being referred to the University Eye Clinic Odense in November 1976 for examination No previous eye symptoms There was nothing unusual in her past medical history A younger sister suffers from epilepsy of unknown aetiology

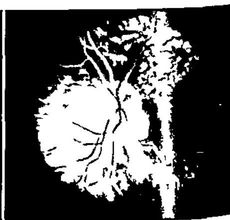
Received June 28 1978



Fig 1

November 19/(Reddish slightly elevated tumour on the left optic disc with cystic detachment and exudation Visual acuity 6 6





Figs 2 and 3

Fig 2 Angiogram arterial phase showing tumour vessels arising from the optic disc

Fig 3 Late angiogram showing massive dyeleakage

Visual acuity was 6/6 emmetropic in both eyes Right eye was normal Fun duscopic examination of the left eye revealed a reddish tumour arising from the lower half of the optic disc and adjacent peripapillary retina A double disc sized cystic detachment extended downwards discrete yellowish exudates at the margins of this detachment were noted Perimetry showed enlargement of the blind spot and a paracentral scotoma this finding corresponding well to those of the ophthalmoscopy The intraocular tension was normal

Fluorescein angiography demonstrated in the retinal arterial phase a well circumscribed mass of small calibre vessels confined to the area around the lower margin of the optic disc Intraretinal leakage of the dye from the tumour was seen in the intermediate arteriovenous phase and late angiograms showed massive dye leakage The calibration of the large central vessels was normal and no evidence of pigment epithelial or retinal abnormality outside the im

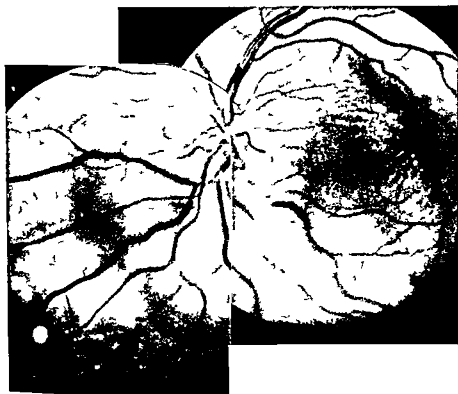


Fig 4

July 1977 Central exudation and cystic detachment progressing Note photocoagula tionsmarks round the periphery Applications on tumour vessels not visible

mediate area of the slightly elevated optic nerve head tumour could be observed

Some progression of the detachment and the exudation were noted during repeated follow up examinations over the next six months Visual acuity remained 6/6 and the visual field defect was stationary She was admitted for a general thorough evaluation in February 1977 No neurologic or other abnormality was found Visual acuity in the left eye had become reduced to 6 9 by June 1977 Further progression of the detachment was noted and the exudation had increased with central oedema and the development of a macular star figure

She was subjected to three applications of argon laser photocoagulation between June and November 1977 The photocoagulation was aimed directly onto the tumour vessels and round the periphery of the lesion After a temporary improvement in visual acuity to 6/6 and apparently some regression in the affection the situation again deteriorated with a gradual reduction in visual



Fig

April 1978 Complete macular star developed Visual acuity reduced to 6/36

icuity to 6/36 and the development of a complete macular star figure in January 1978 The visual acuity was unchanged and the exudation still progressing when last seen in April 1978

Discussion

Three varieties of haemangioma of the optic nerve head have been noted (Henkind & Benjamin 1976)

a) The racemose haemangioma is quite rare but may be part of the Wyburn Mason syndrome with associated midbrain lesions or occurs as an isolated entity The retina usually shows almost no clinical reaction no haemorrhage oedema or exudation The bizarre vessel configurations may approach the macula very closely without interfering with visual acuity Fluorescein angiography is helpful in demonstrating the abnormal arteriovenous connections Quiescent cases show no vascular fluorescein leakage (Henkind et al 1971)

b) The cavernous haemangioma is often asymptomatic and appears to be identical to intraretinal cavernous haemangiomata The condition is not related to von Hippel Lindau s disease The fluorescein angiographic appearance is characteristic with the vascular spaces filling very slowly and often with a fluid level No dye leakage occurs (MacDonald et al 1975)

c) The capillary haemangioma range in appearance from small well circumscribed elevated lesions obscuring part of the disc to large epipapillary vascular masses Bilateral cases of papillary haemangiomatosis have been reported (MacNair et al 1966) but on the whole the condition seems to be almost invariably unilateral The usual type of retinal haemangiomatosis is bilateral in 50% of the cases (Duke Elder 1967) Unlike retinal haemangiomatosis dilatation and tortousity of the feeder vessels do not occur or are not visible in those angiomas arising from or immediately adjacent to the optic nerve head and angiographic evidence of arteriovenous shunting is difficult or impossible to demonstrate (Gass 1974) Johnson et al (1976) found in an ultrastructural study the endothelial cells of the capillary haemangioma to be phenestrated thus providing an explanation of the fluorescein leakage and the extravasated exudate that is characteristic of this tumour About one quarter of patients with capillary haemangiomas have other clinical evidence of von Hippel Lindau s disease (Duke Elder 1967) Visual loss occurs in more than half of the cases of capillary haemangioma and is generally the results of chronic macular oedema Vitreous haemorrhage and retinal detachment may also occur (MacDonald et al 1975)

Photocoagulation is the treatment of choice in retinal haemangiomatosis but is for obvious reasons a risky venture when dealing with papillary juxta papillary lesions The risk of destroying useful central vision is obvious particularly in those located on or surrounding the temporal margin of the optic disc Photocoagulations of selected portions of the tumour may be successful in temporarily reducing the degree of intraretinal and subretinal exudation The long term prognosis for central vision in these papillary and peripapillary tumours is however poor and photocoagulation should not be done in patients with normal visual acuity (Gass 1974) Repeated photocoagulations have not been successful in arresting the exudative changes and vision has decreased from 6/6 to 6/36 over the relatively short period of observation in the reported case

References

Duke Elder S (1967) *System of ophthalmology Vol X* p 741–75[illegible] Kimpton London

Gass J D M (1974) *Differential diagnosis of intraocular tumors* p 26[illegible]–[illegible]6 Mosby St Louis

Henkind P & Benjamin I V (1976) Vascular anomalies and neoplasms of the optic nerve head *Trans ophthal Soc U K 96* 418–423

Henkind P et al (1971) *The retinal circulation* p 263 Harper and Row New York

Johnson F B et al (1976) Angiomatosis retinae *Cancer 38* 204[illegible]–[illegible]056

MacDonald P R et al (1975) Hemangiomas of the optic disc *Canad J Ophthal 10* 30[illegible]–317

MacNair I N et al (1966) Bilateral peripapillary retinae hemangiomas *Arch Ophthal (Chicago)* [illegible] [illegible]–81

Author s address

P Gilbert Nielsen
Dept of Ophthalmology
University Hospital
5000 Odense
Denmark

Landspitalinn Reykjavik Iceland
(Head Kristjan Sveinsson)

HELICOIDAL PERIPAPILLARY CHORIORETINAL DEGENERATION

BY

KRISTJÁN SVEINSSON

A family with helicoidal peripapillary chorioretinal degeneration is described This is a rare bilateral fundus affection – only seven more or less typical cases have been reported in the literature 21 patients from the same family in four generations were examined 10 men and 11 women Seven men and six women showed a helicoidal affection Of this number there were 11 children agen from 4–17 years six were girls of whom three had helicoidal fundus and five were boys of whom two were affected General examination revealed nothing of particular interest We have here a congenital hereditary fundus anomaly or minor malformation in four generations – young people with normal visual acuity who develop with age a clear tendency to invasion of the macular region by a degenerative process This is most dangerous for the visual acuity when the atrophic helicoidal wings lie in or near the macula region

Key words congenital hereditary fundus anomaly – bilateral peripapillar atrophy (Star fish atrophic wings)

In 1939 the author wrote an article in Acta Ophthalmologica (Sveinsson 1939) about curious fundus symptoms which he called chorioiditis areata Later he realized that this terminology was not correct as there was no inflammation In this article the author describes four cases agen from 4–29 years with a curious fundus affection Included in this is a 29 year old mother and her 4 year old son The fundus showed peripapillar chorioretinal atrophy with more or less wide tongue shaped extensions to the periphery having no con

Received May 2 1977

nection with the retinal vessels In none of these cases did the atrophic zones affect the macular region No oedema haemorrhage or inflammatory process was observed The pigment epithelium had disappeared in the helicoidal zones A similar variety of fundus has since been described by others and named differently e g circumpapillaris dysgenesia strati pigmenti retinae (Rubin 1940 1 case) Striate chorioretinitis (Kraffel 1955 1 case) and Helicoid peripapillar chorioretinal degeneration (Franceschetti 1962 1 case) As seen above the names of this fundus affection differ greatly Franceschetti has more than anyone else investigated aberrant fundus affections Therefore in this article the name suggested by him although a long one will be used It is likely that it may often prove difficult to find correct and appropriate terminology for rare diseases or pathological anomalies when everything seems to have been described and classified In many cases there are gaps in our knowledge and in this curious affection there does not appear to be either an inflammatory process or a generation but rather a congenital anomaly or a minor malformation Helicoidal fundus changes are rare Franceschetti (1967) states In fact we have found seven more or less typical cases in the literature which were presented under different names These cases all describe individuals However the author has found four cases including mother and son Hence it was of interest to follow up and carry out research on the family (mother and son) originally reported in 1939

Nearly 40 years have elapsed since then and four generations (21 individuals) come now to be analyzed It should therefore be possible to discover whether we are dealing with a hereditary affection or not In Franceschetti's remarkable book Chorioretinal Heredo Degenerations he writes about helicoidal chorioretinal degenerations and states We have however still no proof that the infantile form of helicoidal peripapillary chorioretinal degeneration is congenital and stationary In this article it is intended to point out that the cases observed in the family in question are congenital hereditary fundus anomalies or minor malformations – apparently stationary infantile forms with a normal visual acuity

With advancing age these patients develop degenerative changes mostly in the central region – endangering the central vision This is of course most marked when the wings (the tongue like radiating atrophies) lie in or near the macula

Results

Examinations have been carried out on 21 persons spanning four generations aged from 4–70 years (Fig 1)

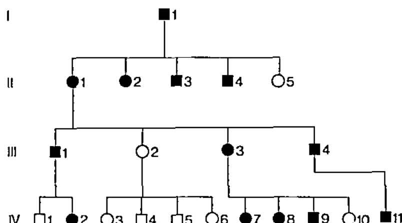


Fig 1

Genealogical tree of a family with dominant transmission of a helicoid peripapillary chorioretinal degeneration through four generations (by Kristjan Sveinsson)

□ = male – normal ○ = female – normal
■ = male helicoid degeneration ● = female helicoid degeneration

I Generation

The ancestor of this family was examined in 1935 (his wife was dead) He was then 70 years old and beginning to see rather poorly He had peculiar chorioretinal foci in both fundi but he died before further examinations could be performed

II Generation

Five of his children were available for examination One daughter A T 68 years old II 1 is the woman examined and reported in 1939 – and also her son then four years old The woman had widely spread helicoidal changes in fundi which lay very close to macula in both eyes Daughter number two 63 years old II 2 had helicoidal fundus vis 6/6 in both eyes Both sons had very clear helicoidal fundus changes One son 61 years old II 3 had poor vision in one eye because of ablatio retinae and helicoidal fundi The vision in the other eye was 6/12 with pigmentary changes in the macula The other son 54 years old II 4 had very poor vision the helicoidal changes were very evident and the macula area was affected by helicoidal foci Daughter number three 51 years old II 5 had normal fundi and vision 6 9 but incipient pigmentary changes were present in the macula in both eyes

III and IV Generation

The children of woman A T (II 1) are as follows Son 48 years old III 1, the one examined when he was four years old with a helicoidal fundus His condition is now vis 6/6 in both eyes but there are primary signs of pigmentary changes in the central region with a mottled appearance both papillae are normal His two children a son 21 years old IV 1 has a normal fundus and his daughter 19 years old IV 2 has inherited her father s fundus (macula normal) Daughter 37 years old III 2 she and her four children IV 3 4 5 and 6 have a normal fundus Daughter III 3 33 years old has a helicoidal fundus + three of her four children two daughters IV 7 and 8 and son IV 9 all have a helicoidal fundus daughter IV 10 has normal fundus son 28 years old III 4 and his son IV 11 both have helicoidal fundus daughter IV 10 has normal fundus son 28 years old III 4 and his son IV 11 both have helicoidal fundi

Fundus examination

These helicoidal fundus changes are similar in all the younger members of the family The description refers to those younger family members with both eyes affected Normal vitreous structure well coloured distinctly defined optic disc Fundus peripapillary atrophy spreading from which can be seen narrow or wide tongue shaped wings extending into the periphery and seemingly reaching further into the nasal retina area In some areas their edges are sharp whereas in other areas smaller or larger notches are observed Choroidal veins can be clearly seen in the periphery and at the edges of these wings Elsewhere the shapeless white scleral area can be seen over the most part of the wings where the pigment epithelium has almost or completely disappeared Spreading of slight pigmentation is sometimes to be seen over these spots The retinal vessels which in appearance seem to be quite normal lie over these spots in some areas without following them

In most of the young people the fundus is otherwise normal except in some areas close to the edges adjoining the atrophic wings where the colour is a rather darker red It is therefore probable that the pigment layer that should have covered the atrophic spots could not reach any further and has accumulated into a double layer It could also partially be a contrast phenomenon against the white area Niveau changes do not seem to exist Oedema exudates and haemorrhages are not found in these fundi Vision is nearly normal in most of the young people a few have a mild astigmatism Serious myopia does not occur Visual field for white is normal but defects appear in the visual field corresponding to the fundus damage Colour vision Stilling Ishihara adjustment is normal Dark adaptation is normal

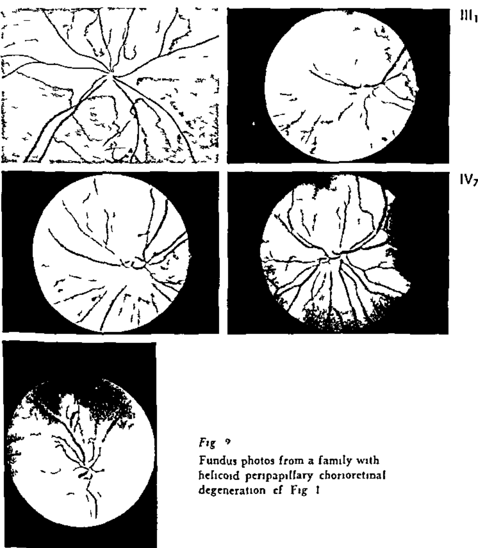


Fig 9
Fundus photos from a family with helicoid peripapillary chorioretinal degeneration cf Fig 1

This description of the fundus applies to both young and old persons However with age the young patients start to develop a degenerative stratumh pig menti retinae chiefly in the central region of the eye

The patient A T 68 years old II 1 (Fig 2) has been followed up for the longest period At the time of the first examination in 1936 she was 26 years old and widely spread helicoidal changes were found in both fundi very close to macula

Vision was then 6/12 in the right eye with – 2 0 sph and 6 9 in the left eye with – 1 0 sph When the patient was close to fifty years of age she lost the central vision and was then only able to count fingers eccentrically in front of the eye The atrophic process had greatly progressed in the macular and peripapillary regions Since then the fundus anomalies have been stationary Both brothers have developed reduced vision. A few years ago the elder brother 61 years old II 3 had 6 9 vision in the right eye but this has now fallen to 6/12 with pigment changes in the macula He lost sight in the left eye after an ablatio retinae and had helicoidal fundus changes The younger brother II 4 54 years old has always experienced difficulty in reading because of the extensive helicoidal fundus changes It is remarkable that the fault in the pigment epithelium near the edges of the atrophic wings is most noticeable in the central region of the eyes in all three siblings The 43 years old son of A T, III 1 seems to be developing a degeneration of the pigment epitheepithelium in the central region

Discussion

Twenty one persons were examined 10 men and 11 women as shown in Fig 1 The four generations – seven men and five women were found to have similar congenital helicoidal fundus affection Of this number there were 11 children aged from 4–17 years six girls of whom three had a helicoidal fundus and five boys of whom two were affected This is therefore a clear hereditary case with an autosomal dominant mode of transmission Dr L Magnusson an eye specialist in Akureyri has come across one family living in North East Iceland where this curious alteration of the fundus occurs in many of the family members In all likelihood this is the same family

What could be the cause for this strange fundus anomaly? From a genetic point of view this anomaly or malformation is clearly distinct from other *affections of the fundi* Many sclerotic diseases *occur in the chorioidea* which cause atrophy and destruction of the pigment epithelium e g in ischaemic atrophy choroidal sclerosis and choroidal atrophy These diseases often appear with massive pigmentary proliferative which is spread over fundus and are then progressive This occurs more often in adults and is therefore not similar to helicoidal affection Judging by the shape of the atrophic fundus spots, one could suggest insufficiency or defects in one or more branches of of the arteriae ciliares posticae breves which would not only cause defects in the chorioidea, but would also cause secondary damage in the pigmentary epithelium and retinae. This is not a case of inflammatory or obvious degenerative af

fection in young people but represents either a congenital inherited anomaly or a malformation resulting from a development disorder in the formation of the chorioidea or stratum pigmenti retinae or lack or defect or aplasia in arteriae ciliares posticae breves An autosomal dominance is here the usual mode of transmission

All hereditary diseases of course depend initially on the development of a pathogenic gene from a normal gene The members of this family who are apparent carriers of the same pathological gene present a similar clinical picture and gene expression The gene has a high penetrance and a high degree of expression and in these cases the pathological gene is expressed only in part of the tissue that it is generally associated with

References

Franceschetti A François J & Babel J Chorioretinal Heredo Degenerations p 479

Franceschetti A (1962) A curious affection of the fundus oculi Helicoidal peripapillar chorioretinal degeneration Its relation to pigmentary paravenous chorioretinal degeneration *Docum ophthal 16* 81–109

Kraffel G (1955) Beitrag zur chorioretinitis striata *Klin Mbl Augenheilk 127* 664–669

Rubino A (1940) Su una particulare anomalia bilaterale e simmetrica dello strato pigmentato retinico *Boll Ocul 19* 318–322

Sveinsson Kr (1939) Chorioiditis areata *Acta ophthal (Kbh) 17* 73–79

Author s address

Kristjan Sveinsson
Öldugotu 9
Reykjavik
Iceland

Department of Ophthalmology University of Oslo Rikshospitalet Norway
(Head Thore L Thomassen)

AXIAL LENGTH OF THE EMMETROPIC EYE AND ITS RELATION TO THE HEAD SIZE

BY

JON S LARSEN

The results of the ultrasonographic measurements of the ocular components in 40 emmetropic eyes from men of nearly the same age are presented and the possible relation between the axial eye length and the head size is discussed A significant correlation was found between the axial eye length and the head circumference ($P < 0\ 001$) between the axial eye length and the head length ($P < 0\ 01$) and between the axial eye length and the head breadth ($P < 0\ 02$) A significant correlation was also found between the axial eye length and the height of the subjects ($P < 0\ 01$)

It is suggested that such a correlation can explain not only the wide individual range of axial eye lengths in emmetropic eyes but also the existing difference in axial eye length between the sexes

The mean eye length was 23 82 mm in 40 men and 23 02 mm in 10 women both sexes were of the same age (20–30 years)

Key words ultrasonography – axial eye length – emmetropia – head size.

The average value of the axial length of emmetropic eyes is given in most recent investigations as lying between 23 2 and 23 5 mm (Gernet 1964 196 François & Goes 1971 1977 Karantinos et al 1974) In the majority of the studies a considerable variation in axial length of emmetropic eyes is found (Gernet 1964 1967 Fridman & Savitskaya 1966 François & Goes 1977) and this can be more than 5 0 mm (François & Goes 1971) There seems also to be agreement that the axial eye length is on average greater for men than for women (Gernet 1964 Nover & Grote 1965 Fridman & Savitskaya 1966 Larsen 1971 François & Goes 1977)

Received September 8 1978

A review of the literature on this topic does not appear to disclose any information which can explain the difference in axial length between the sexes or the individual variation No studies seem to have been done either to reveal to what extent there exists a correlation between the head size and the axial length of the emmetropic eye The object of this work was therefore to further clarify these relationships by examination of emmetropic individuals of the same age and sex

Material

The material included 50 emmetropic eyes (refraction between –0 5 and +0 5 dptr) of subjects between 20 and 300 years of age All the subjects 40 men (average age 26 4 years) and 10 women (average age 24 7 years) had 20/20 vision in the right eye which was examined

Methods

The head length was measured between the most projecting part of the back of the head opisthocranion and glabella The head breadth was measured between the two most protruding points above porus acusticus externus The head circumference was measured all round the most projecting part of squama frontalis and the most projecting part of os occipitale

All measurements of the eye were performed under cycloplegia Cyclopentolate hydrochloride 1% (Cyclogyl®) was instilled twice at intervals of about 10 min 30–45 min before a streak retinoscopy and the examinations were carried out. The Javal Schiøtz ophthalmometer (Haag Streit) was used to determine the corneal radius The results were the average value between the horizontal and vertical radius The horizontal corneal diameter was determined from a colour photograph (magnitude at limbus corneae 1 1)

The depth of the anterior chamber (including corneal thickness) axial thickness of the lens and the vitreous length were measured ultrasonographically The ultrasound apparatus used in the research consisted of an A scan instrument Kretztechnik model 7200 MA and of a 10 MHz/5 mm transduced (KM 10/5 K) Small swim goggles with perforated glasses were employed for the immersion technique In order to avoid echographic disturbances from cilia and eyelids a small blepharostat (model Barraquer) was used The echograph was calibrated in microseconds and the calibration checked before each new subject was examined. In order to calculate the ocular distance in millimeters we used the following ultrasound velocities 1532 m/sec in the camera anterior and the vitreous and 1641 m/sec in the lens (Jansson & Kock 1969) The distance of the ocular parameters were measured from a polaroid photo with a precision compass (Helios) including an odometer graduated in 1/20 mm The equipment setting and amplification were those used for obtaining a maximal amplitude echo of a detached retina An average of five measurements with an optimum re

Department of Ophthalmology University of Oslo Rikshospitalet Norway (Head Thore L. Thomassen)

AXIAL LENGTH OF THE EMMETROPIC EYE AND ITS RELATION TO THE HEAD SIZE

BY

JON S LARSEN

The results of the ultrasonographic measurements of the ocular components in 40 emmetropic eyes from men of nearly the same age are presented and the possible relation between the axial eye length and the head size is discussed A significant correlation was found between the axial eye length and the head circumference ($P < 0.001$) between the axial eye length and the head length ($P < 0.01$) and between the axial eye length and the head breadth ($P < 0.05$) A significant correlation was also found between the axial eye length and the height of the subjects ($P < 0.01$)
It is suggested that such a correlation can explain not only the wide individual range of axial eye lengths in emmetropic eyes but also the existing difference in axial eye length between the sexes.
The mean eye length was 23.82 mm in 40 men and 23.0_ mm in 10 women both sexes were of the same age (20-30 years)

Key words ultrasonography – axial eye length – emmetropia – head size.

The average value of the axial length of emmetropic eyes is given in most recent investigations as lying between 23.2 and 23.5 mm (Gernet 1964 1967 François & Goes 1971 1977 Karantinos et al 1974) In the majority of the studies a considerable variation in axial length of emmetropic eyes is found (Gernet 1964 1967 Fridman & Savitskaya 1966 François & Goes 1977) and this can be more than 5.0 mm (François & Goes 1971) There seems also to be agreement that the axial eye length is on average, greater for men than for women (Gernet 1964 Nover & Grote 1965 Fridman & Savitskaya 1966 Larsen 1971 François & Goes 1977)

Received September 8 1978

Table I
Correlations between the eye length the various head dimensions and the subjects height

No and sex of subjects	40 Males
	Axial eye length
Head circumference	r = +0 6055 t = 4 6899 $P < 0\ 001$
Head length	r = +0 4703 t = 3 2851 $P < 0\ 01$
Head breadth	r = +0.3752 t = 2 4953 $0\ 01 < P < 0\ 02$
Height of subjects	r = +0 4457 t = 3 0692 $P < 0\ 01$

We found a significant correlation between the head circumference and the axial eye length ($P < 0\ 001$) Fig 1 Table V There was also a significant correlation between the head length and the axial eye length ($P < 0\ 01$) between the head breadth and the axial eye length ($P < 0\ 02$) and between the height and the axial eye length ($P < 0\ 01$) In this material of 40 men there was an important correlation between the head circumference and the height of the subjects (r = +0 5555 $P < 0\ 001$)

Discussion

The relationship between the different ocular parameters found in this investigations (Table III) was in agreement with findings from former studies of emmetropic eyes Between the corneal radius and the axial eye length Sorsby et al (1957) obtained a correlation coefficient of r = +0 877 and François & Goes (1971) determined a correlation factor of r = +0 64 in emmetropic eyes Between the depth of the anterior chamber and the axial eye length François & Goes (1977) determined a correlation factor of r = +0 4684 We

obtained also a rather important positive correlation between the corneal radius and the axial eye length (r = +0 4569) as well as between the depth of the anterior chamber and the axial eye length (r = +0 5424) We measured a significant negative correlation between the depth of the anterior chamber and the thickness of the lens (r = −0 2808 $P < 0\ 05$) Other authors could also demonstrate an important negative correlation between the depth of the anterior chamber and the thickness of the lens in emmetropic eyes (Delmarcelle et al 1969 1970 r = −0 60 François & Goes 1977 r = −0 5770) In our study the axial length and the thickness of the lens varied independently (r = −0 1200 $0\ 20 < P < 0\ 50$) François & Goes (1971 1977) after comprehensive studies of emmetropic eyes also came to the same conclusion Our material seems therefore although it was relatively small to be representative with respect to the relationship between the ocular components

We could demonstrate a rather important difference of 0 80 mm between the mean eye length in men (23 82 mm) and in women (23 02 mm) Other results in the literature could also demonstrate a longer eye length of 0 60–0 80 mm in men as compared to women (Gernet 1964 0 60 mm Nover & Grote 1965 0 60 mm Fridman & Savitskaya 1966 0 77 mm Fridman 1968 0 70 mm François & Goes 1977 0 80 mm) The authors agree that the variations between the eye lengths in emmetropic eyes are considerable and can be more than 5 0 mm (Gernet 1964 3.55 mm Fridman & Savitskaya 1966 3 1 mm Franceschetti & Luyckx 1967 3 0 mm François & Goes 1971 5 25 mm present study 3 59 mm (men))

In the literature there seems to be no information which can explain these circumstances We could however in this investigation demonstrate a significant correlation between the axial length in emmetropic eyes and the head size It is suggested that such a correlation can explain not only the wide individual range of axial eye lengths in emmetropic eyes but also the existing difference in axial eye length between the sexes

References

Delmarcelle Y Collignon J & Luyckx J (1969) La profondeur de la chambre antérieure de l oeil et ses facteurs constituants *Bull Soc belge Ophtal 153* 447–453

Delmarcelle Y Collignon J & Luyckx J (1970) Role de la cornee et du cristallin sur la biometrie de la chambre anterieure du sujet normal *Arch Ophtal (Paris) 30* 291–300

Franceschetti A Luyckx J (1967) Etude de l effet emmétropisant du cristallin par la methode d echographie ultrasonique *Ann Oculist 200* 177–190

rançois J & Goes F (1971) Echographic study of the lens thickness as a function of the axial eye length in emmetropic eyes of the same age In Bøck J and Ossoi nig K Ed *Ultrasonographia medica* pp 531–538 Wiener Med Akademie Vienna
rançois J & Goes F (19//) Ultrasonographic study of 100 emmetropic eyes *Ophthalmologica 1/5* 321–327
ridman F E (1968) Bedeutung der Ultraschallbiometrie beim Studium der anatomo optischen Elemente der Augen verschiedener Refraktionen In Vanysek J Ed *Diagnostica ultrasonica in ophthalmologia* pp 259–263 Universita J Purkinje Brno
ridman F E & Savitskaya N F (1966) Ultrasound biometry in the study of the anatomo optical elements of the eye in myopia *Vestn Oftal /9* 30–33
ernet H (1964) Über Achsenlange und Brechkraft emmetroper lebender Augen *Graefes Arch Ophthal 166* 424–431
ernet H (1967) Ultraschall Biometrie des Auges *Klin Mbl Augenheilk 151* 853–871
ansson F & Kock E (1962) Determination of the velocity of ultrasound in the human ens and vitreous *Acta ophthal (Kbh) 40* 42–433
arantinos E Papacharalampous G Theodossiadis G & Velissaropoulos P (1974) Biométrie oculaire par echographie A *Arch Ophtal (Paris) 34* 581–586
arsen J S (1971) The sagittal growth of the eye I IV *Acta ophthal (Kbh) 49* 239–269 427–440 441–453 873–886
over A & Grote N (1965) Über die Bestimmung der Achsenlange des menschlichen Auges mit Ultraschall am Lebenden *Graefes Arch Ophthal 168* 405–418
Rauber Kopsch (Kopsch F Ed) (1947) *Lehrbuch und Atlas der Anatomie des Menschen Allgemeines Skeletsystem Muskelsystem* pp 247–250 Georg Thieme Leipzig
orsby A Benjamin B Davy J B Sheridan M & Tanner J M (1957) Emmetropia and its aberrations A study in the correlation of the optical components of the eye *Med Res Counc Spec Rep Ser No 293* H M S O London
Van der Heijde G L & Stilma J S (19/6) Biometric und Aphakie *Klin Mbl Au enheilk 169* 289–293

Author s address
on S Larsen,
illehagveien 68
310 Blommenholm
Norway

ACTA OPHTHALMOLOGICA VOL. 57 1979

The Institute of Clinical Genetics (Head: M. Hauge)
University of Odense and
The Department of Ophthalmology (Head: E. Gregersen)
Rigshospitalet, University of Copenhagen

REFRACTION IN ADULT WEST GREENLAND ESKIMOS

A Population Study of Spherical Refractive Errors Including Oculometric and Familial Correlations

BY

P. H. ALSBIRK

Refractive error (RF) was determined using subjective technique and Snellen chart in a West Greenland Eskimo population in the district of Umanaq. Both eyes of 483 and one eye of 25 persons were refracted in age groups above 15 years. Spherical equivalents of any astigmatic errors were included in RF values.

Emmetropic excess and *myopic skewness* of RF distributions were found. A significant trend towards hypermetropia with *age* was found in women, not in men. Myopia was no rarity: 6.3 % of male eyes and 4.2 % of female eyes showed myopia stronger than -2.0 diopters. Moderate myopia -5 to -1 d. was found in 13.3 % and higher myopia in 0.8 % of the persons.

RF and ocular dimensions showed strong correlation for axial and vitrous length (r = -0.45) only and weaker correlations for chamber depth and thickness of lens and cornea. No association was found between RF and height.

Family variations of RF were compared with those of ocular dimensions. Judged by child-parent relationships a much lower heritability was found in RF (h = 0.14) than in axial length, corneal curvature and chamber depth (h = 0.76, 0.64 and 0.56 respectively). However, sibs showed a higher RF similarity than child-parent relationships, suggesting an influence of common familial environment upon RF, although theoretically genetic (dominance variance) factors may also be responsible.

Presented in part at The Second International Conference on Myopia, May 1978, Yokohama, Japan.

Received July 14th, 1978.

Recent epidemiologic and genetic studies strongly emphasize the *importance of environmental factors* to human refraction The present family study supports such ideas Refraction seems to be highly influenced by environment in spite of the mainly genetic determination of relevant ocular dimensions

Key words refractive error – myopia – ocular dimensions – height – age/sex – Greenland Eskimos – heritability – genes/environment

Through most of this century since the days of Steiger (1913) the majority of human refractive errors have been considered to be genetically determined As late as 1972 Sorsby expressed his concept of refraction based on several studies by the following words The traditional emphasis on environmental factors as productive of refractive errors finds no support in the detailed studies of to day As to low myopia developed during growth Goldschmidt (1968) like most other modern authors shared this opinion However he pointed out that a rare late myopia exists which is caused by extensive fatiguing near work

In fact the many low or simple myopes of civilized populations were considered to result from relaxed natural selection in such communities (e g Post 1962) against the nearly non existing myopia in primitive populations Thus the surveys of Holm (1937) in Gabon Negroes and Skeller (1954) in pure East Greenland Eskimos were taken to support a genetic hypothesis

However this mainly genetic concept of refractive errors has not been unopposed through the last decades e g Lindner (1944) In Japan the old European theories of school myopia have currently received strong support although the pathogenetic mechanism has been the object of much dispute, e g Sato (1957) and Otsuka (1967) Recently epidemiologic and genetic surveys in North American Eskimos have shown that the traditional genetic concept of refractive errors has to be modified An adolescent epidemic of myopia has been independently reported in Alaskan and Canadian Eskimos by Young et al (1969) Morgan & Munro (1973) and Alsbirk & Forsius (1973) Furthermore a number of family studies have shown that the heritability of refractive error is low about 25 % cf the survey by Spivey (1976) based on reports by Sorsby et al (1966) Young & Leary (1972) and Hegmann et al (1974) as well as the Eskimo family study by Morgan et al (1975)

The present population study of refractive error in adult Greenland Eskimos contributes to the epidemiology and genetics of this important visual parameter Relevant oculometric findings are included

Material and Methods

The basic material of the present study was 931 Greenland Eskimos living in the district of Umanaq in 1969 They constituted 95% of the two following census population groups 652 town inhabitants above 7 years and 278 persons in the 8 villages aged 40 years or more (Alsbirk 1974) This sample was examined by optical pachymetry in 1969 The *adults* aged 15 years or more were reexamined in 1972 using *ultrasound oculometry* and in the summer of 19 2 using Javal Schiøtz keratometry and *subjective refraction* in front of Snellen's chart at 6 m cf Alsbirk (1975) The refractions were made in cooperation with ophthalmic assistant Ida Alsbirk who has several years experience in prescription of glasses in Greenland.

At the *refraction survey* one village (Satut with 55 persons) was excluded due to lack of time. Out of the remaining 720 adults 533 were examined. Apart from persons absent (31) moved (53) dead (29) or diseased (4) only 40 persons (= 7%) of the target group were not examined, due to non appearance (32) or refusal (8)

Corneal and subjective astigmatism will be described in a later publication. In the present paper the *refractive error (RF)* is given in spherical equivalents i.e. sphere + ½ x signed cylindrical refractive error When possible the average of both eyes was taken to represent the individual Cycloplegia was not used as we were not prepared to do angle closure glaucoma surgery possibly indicated in some shallow chamber individuals of this high risk population (Alsbirk 1976)

Thus RF was estimated in 505 of the 533 persons examined in both eyes of 480 and in one eye of 25 persons A total of 75 eyes (7%) could not be refracted, mostly due to poor visual acuity or lack of cooperation Visual acuity distributions including rate of blindness as well as use and need of spectacle corrections will be described elsewhere

Results

Distributions of refractive errors (RF) are illustrated in Fig 1 The well known unimodal non Gaussian pattern was found ($P < 0.001$ by χ^2 goodness of fit tests) An excess around emmetropia was found in both sexes but a test of kurtosis was significant in men only ($P < 0.001$) In both sexes a significant myopic skewness was found $P < 0.02$ in men and $P < 0.001$ in women.

Age variation of RF was insignificant in men but showed a positive regression coefficient in women in whom an increase of 1 2 diopters from age 20 to 70 was found cf Table I and Fig 2 Thus a highly significant *sex difference* appeared in persons more than 40 years old the women being 0.73 d more hypermetropic than men In younger generation an opposite insignificant trend was found

RF of right and left eyes could be compared in the 483 persons of Table I No significant side difference was found (average 0 02 d) Right and left eyes showed a high mutual similarity (correlation coefficient r = +0 85) Thus about 90 % of all persons refracted showed anisometropia below 1 0 d 95 % below 1 5 d and 99 % below 3 d while only 4 persons had higher anisometropia about 5 d

Rates of myopia are given in Table I as percentual frequencies according to two simple arbitrarily chosen principles of truncation About 6 % of male and 4 % of female eyes showed myopia stronger than –2 0 d (95 % confidence intervals Men 6 3 ± 3 2 % women 4 2 ± 2 5 % each person counted once)

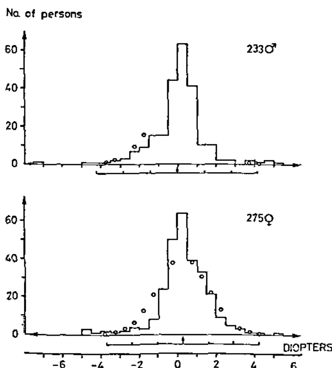


Fig 1

Refractive error (RF) distributions of adult Greenland Eskimos based on 991 eyes of 503 persons Spherical equivalents are given, averaged in bilaterally refracted persons The mean values ± 3 SD are shown below the histograms The circles indicate expected nos per half diopter class according to a Gaussian distribution

Table I

Refractive errors (RF) in 483 Greenland Eskimos bilaterally refracted according to age sex and percentage of myopic eyes

Age	No and sex of persons	RF as average of both eyes (diopters)				Myopia in per cent RF more negative than	
		mean	SD	median	range	−2.0 d	0.0 d
15–19	20 M	−0.4	1.7	0.0	−3.5 to +5.0	*15*	*43*
20–29	57 M	+0.1	0.6	+0.1	−1.5 to +2.0	–	*27*
30–39	57 M	+0.2	0.9	0.0	−2.4 to +3.8	*2.7*	*24*
40–49	53 M	−0.3	1.6	0.0	−7.4 to +2.6	*9.4*	*43*
50–59	58 M	−0.0	1.4	−0.2	−2.3 to +4.1	*2.6*	*55*
60+	37 M	−0.2	1.4	+0.1	−4.0 to +2.0	*10.8*	*46*
15–39	94 M	+0.07	1.06	0.0	−3.5 to +5.0	*4.3*	*29.3*[1]
40+	128 M	−0.18	1.50	−0.1	−7.4 to +4.1	*7.8*	*45.4*[2]
15+	222 M	−0.0	1.34	0.0	−7.4 to +5.0	*6.3*	*40.3*[3]
15–19	29 F	−0.2	1.0	−0.2	−2.5 to +2.5	*1.7*	*48*
20–29	37 F	−0.2	1.3	0.0	−4.8 to +1.5	*8.1*	*39*
30–39	48 F	−0.1	0.8	0.0	−2.4 to +1.5	*4.2*	*41*
40–49	56 F	+0.2	1.0	+0.3	−5.0 to +1.8	*1.8*	*34*
50–59	43 F	+0.5	1.6	+0.5	−5.0 to +3.6	*5.8*	*29*
60	48 F	+1.0	1.6	+1.2	−4.5 to +5.0	*4.2*	*15*
15–39	114 F	−0.14	1.01	0.0	−4.8 to +2.5	*4.8*	*42.1*[1]
40	147 F	+0.55	1.45	+0.5	−5.0 to +5.0	*5.7*	*26.2*[2]
15+	261 F	+0.25	1.30	+0.25	−5.0 to +5.0	*4.2*	*33.1*[3]

Sex variation 1) χ^2 = 3.1 n.s 2) χ^2 = 13.7 $P < 0.001$ 3) χ^2 = 2.4 n.s

Age variation estimated by linear regression coefficient (b)

In 222 M b = −0.004 diopters per year s_b = 0.006 n.s

261 F b = +0.024 diopters per year s_b = 0.005 $P < 0.001$

Frequencies of *all negative RF values* are included in Table I for the purpose of comparison with Young et al (1969 Alaska Eskimos showed the following per cent of myopes age 16–20 59%, 21–25 88%, 26–30 43%, 31–40 23% 41–50 4% and 51 and above 0% right eyes only cycloplegically refracted) In the Canadian Eskimo survey (Morgan & Munro 1973) *moderate myopia* was taken as 1 to 5 diopters of myopia. In the present survey we found $-5.0 \leq RF \leq -1.0$ d in 9.4% 9% and [illegible]% of the pooled age groups 15–19 20–29 and 30–39 years respectively (average 12.0%) above 40 years the rates 11% 19% and 14% were found in age groups 40–49 50–59 and 60+ years (average 14.2%) The fairly high estimate in 15–19 year old

dolescents is based on only 12/49 persons but approximates the value given by Mor
an & Munro (1973) about 25 % *High myopia* RF more negative than −5 0 was
ound in one eye of 2 men (−10 and −5 5 d) and in one eye of 2 women (−6 25 and
0 d) i e in one eye of 0 8 % of the persons bilaterally refracted

PF and ocular dimensions could be correlated in a subsample of 325 persons in whom all oculometric variables had been measured (cf Alsbirk 1977) As the 137 men and 188 women showed nearly identical values pooled estimates are given Between RF and ocular dimension the following correlation coefficients were found (in ranked list) Vitreous length −0 45** axial length r = −0 44** anterior chamber depth (as age and sex independent deviation score) −0 18 * radius of corneal curvature +0 05 corneal thickness +0 12* (cf Alsbirk 1978) lens thickness +0 15* (−0 06 in men, +0 39 * in women) The asterisks indicate significance at the 0 05 or 0 01** level Thus well known more or less pronounced associations between ocular dimensions and RF were found

PF and height could be analysed in a subsample of village inhabitants aged 43 years or more in whom height had been measured 9 years earlier by Littauer et al (1976) Insignificant correlations were found r = + 0 20 in 66 men and r = + 0 08 in 65 women Thus no association between body height and myopia was suggested in this group

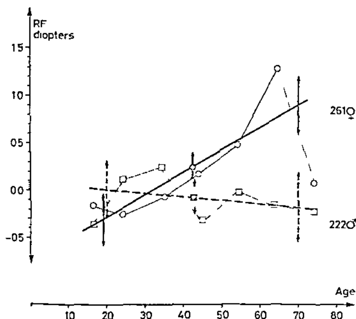


Fig 9

RF and age in adult Greenland Eskimos based on 483 persons bilaterally refracted Decade mean values (males □ females ○) and linear regressions with 95 % confidence limits at mean ages and arbitrary extreme ages are shown

Table II

Family variation of age and sex independent ocular dimensions corneal radius of curvature (CR) axial length (AL) chamber depth (ACD) and spherical refraction error (RF) Estimates are given as regression coefficients (b) intrapair correlation coefficients (r_I) with standard errors (SE)

Family relationship	No of pairs	CR		AL		ACD		RF		Maxim. genetic expect.
		b	SE	b	SE	b	SE	b	SE	b
Child father	52	0.26	0.12	0.46	0.15	0.42	0.11	0.13	0.11	0.5
Child mother	107	0.36	0.09	0.34	0.11	0.21	0.08	0.05	0.08	0.5
Child parent	159	0.32	0.07	0.38	0.09	0.28	0.07	0.07	0.06	0.5
Child midparent	49	0.47	0.17	0.55	0.22	0.74	0.18	0.02	0.17	1.0
Husband wife	108	0.02	0.10	0.05	0.10	−0.09	0.10	0.03	0.09	0.0
		r_I	SE	r_I	SE	r_I	SE	r_I	SE	r_I
Brothers	28	0.2[illegible]	0.18	0.[illegible]0	0.18	0.58	0.13	0.39	0.16	0.5
Sisters	51	0.29	0.13	0.19	0.14	0.40	0.12	0.27	0.13	0.5
Sibs	160	0.20	0.08	0.25	0.07	0.43	0.06	0.25	0.0	0.5

Family variations of RF and ocular dimensions are shown in Table II Due to age and sex variation of RF a transformation of dioptric error to age and sex independent deviation score was performed prior to analyses For the purpose of comparison three relevant oculometric variables axial length corneal radius of curvature and anterior chamber depth were transformed correspondingly Detailed procedures are described elsewhere (Alsbirk 1975)

The subgroups of the family material showed conspicuous variations which however must be considered on the background of the large standard errors even in the pooled groups of Table II

Husband wife pairs showed no mutual similarity and thus no assortative mating seems to occur First degree relationships showed largely the same fairly high level of familial oculometric resemblance in child parent as well as in sib comparisons In contrast to this pattern the *refractive error* analyses showed *no significant child parent correlation* but a significant sib correlation Al though the standard errors call for reservation the finding suggests that certain influences upon RF tend to make sibs more alike in refraction than children and parents Two explanations seem possible common familial *environmental* factors during childhood influencing adulthood refraction or a *genetic* factor the so called dominance variance component Interpretation will be discussed below

Discussion

Recent changes of refraction in Eskimos has been a challenge to traditional mainly genetic concepts of the human refractive state The stability through generations of refractive error distributions has now lost global validity at least in rapidly changing communities In the *Alaska and Canada Eskimo* surveys the elderly adults had grown up as arctic nomads in small hunting communities without schooling while after world war II children and adolescents as a rule have attended school regularly The present study was made among *West Greenlanders* living in a hunting and fishing community The development through this century has run a fairly quiet course here compared to the violent changes in Alaska and Canada West Greenland has been under Danish administration for about 250 years For more than 100 years a written Eskimo language has been taught in schools and analphabetism is practically eradicated although the general population does not read much All adult age groups of the Umanaq Eskimos were found to be fairly often myopic According to earlier clinical observations this should not be a great surprise (Bertelsen 1940 Lawætz 1949 Skeller 1949) although population studies have been lacking On the other hand the isolated Angmagssalik district in *East Greenland* has a special historical backgrund The population here was colonized as late as 1894 and no written East Greenland language exists their dialect being very unlike that of the West Greenland communities It has now been made completely clear that the very rare myopia found by Skeller (1954 no eye more myopic than -1 25 d among 1100 eyes examined) is not a general or permanent characteristic of all Eskimos

The present study did not include children and was made without cycloplegia At least *two additional studies* ought to be done in Greenland in the near future a repeated investigation of Skeller s material from Angmagssalik where much has changed since 1950 now supplemented with a new generation Further a cycloplegic population survey in children and adolescents of one of the greater towns (and school centers) of West Greenland in order to see if there is an adolescent epidemic of myopia in such more rapidly developing communities

Surprisingly the age variation differed between men and women in the present study The hypermetropic trend with increasing age corresponds to general clinical experience cf Slataper (1950) but was found in women only A recent population study in Israel revealed a similar preponderance of females among hypermetropes and of males among low myopes above the age of 40 cf Hyams et al (1977) Socioeconomic factors were not included in the present analyses However in a subsample of men having some education (16 teachers clerks telegraphists) no significant excess of myopia was found

Family variations of refractive error and its determining oculometric variables *have only been analysed in a few earlier studies* The preponderance of genetic factors in European concepts of refraction has largely been based on selected pedigrees and twin studies but important sources af bias have rarely been recognized As the present analyses show the methods of quantitative genetics are useful but at least as crude as the standard errors are large However important family studies recently published agree with the present trends Thus Hegman et al (1974) found a low refractive error parent offspring resemblance in their randomly selected material of 181 families which gave a heritability estimate of h ± sr = 0 24 ± 0 05 Thus a low heritability of spherical refractive errors was found compared with e g interpupillary distance (h = 0 65 ± 0 05) or corneal power (h = 0 89 ± 0 05 Mash et al 1975) Based on the present child parent regressions of Table II heritability of refractive error was estimated at h = 0 14 ± 0 12 while relevant ocular dimensions showed higher values corneal curvature radius h = 0 64 ± 0 14 axial length h 0 76 ± 0 18 and anterior chamber depth 0 56 ± 0 14

A relatively higher resemblance between sibs than between parent and offspring was a prominent finding in the present refractive error analyses and similar results appeared in Sorsby et al s study (1966) and particularly in the Eskimo family study performed by Young et al (1969) On the other hand, sibs from Washington State studied by Young (1958) showed a low mutual resemblance (partial r = 0 14) Such discrepancies are not easily explained. Although dominance variance through the sharing by sibs of one quarter of all pairs of alleles may be a significant source of higher resemblance in sibs environmental influences bound to the family conditions may also exist Such *common familial environmental factors* may be strong in some and weak in other populations and periods Twin studies especially may give such inflated levels of resemblance which will only be recognized if several monozygotic *twins reared apart can be analysed and this is very rare* Probably sibs and half sibs would be more useful in an attempt to separate such common familial environment and dominance effects in human genetics cf Li (1977)

However in spite of such uncertainties it is now clear that the conclusions based on twin studies like that of Sorsby et al (1962) have not been confirmed neither by their own (1966) nor by other larger studies of family variations of refractive error The static genetic concept of refraction has to be modified Once again environmental factors probably related to near visual demands of school age seem to be important (e g Tscherning 1883 Lindner 1944 Sato 1957 Morgan et al 1975 Young 1977) As a clinical consequence of such changing views Oakley & Young (1975) treated a group of 226 slightly myopic children with +1 5 d bifocal add to distance correction Compared with the

annual progression of -0 5 d in the control group the bifocally treated children showed a negligible average progression (-0 03 d per year) A few other reports have appeared e g Bedrossian (1971) who succesfully used atropine mono cularly to stop myopic progression and Kelly et al (1975) who achieved arrest of myopia using bifocals (add +1 5) and phenylephrine 5 % However such reports as well as the current views of Japanese ophthalmology have generally *been received with great scepticism Probably a new era reaccepting the im* portance of environmental influence in myopia pathogenesis is now at the beginning due to results of epidemiologic genetic and experimental research through the last ten years

In conclusion the present study has shown that myopia is fairly frequent in all adult ages in West Greenland Eskimos who have all attended school and use a written language No adolescent epidemic of myopia was found in the district population examined However age variation showed a sex difference elderly women being more hypermetropic than men Family variations in adults showed almost no resemblance between the refraction of children and parents but a somewhat higher correlation between sibs The results are cautiously in terpreted as a support to the redeveloped concepts of environmental influence upon human refractive state Refraction shows a lower heritability than relevant ocular dimensions

Acknowledgments

Supported by grants from Fabrikant Einar Willumsens Mindelegat the Danish Com mittee for Prevention of Blindness the Danish Medical Research Council the Commis sion for Scientific Research in Greenland and the Danish Hospital Foundation for Medical Research Region of Copenhagen the Faroe Islands and Greenland

References

Alsbirk P H (1974) Anterior chamber depth in Greenland Eskimos I A population study of variation with age and sex *Acta ophthal (Kbh)* 52 551-564

Alsbirk P H (1975) Anterior chamber depth and primary angle closure glaucoma II A genetic study *Acta ophthal (Kbh)* 53 436-449

Alsbirk P H (1976) Primary angle closure glaucoma Oculometry epidemiology and genetics in a high risk population *Acta ophthal (Kbh)* 54 *Suppl* 127 1-31

Alsbirk P H (1977) Variation and heritability of ocular dimensions A population study among adult Greenland Eskimos *Acta ophthal (Kbh)* 55 443-456

Alsbirk P H (1978) Corneal thickness I Age variation sex difference and oculometric correlations *Acta ophthal (Kbh)* 55 95-104

Alsbirk P H & Forsius H (1973) Anterior chamber depth in Eskimos from Greenland Canada (Igloolik) and Alaska (Wainwright) A preliminary report *Canad J Ophthal 8* 265–269

Bedrossian R H (19 1) The effect of atropine on myopia *Ann Ophthal 3* 891–897

Bertelsen A (1940) Grønlandsk medicinsk statistik og nosografi *Meddelelser om Grønland 11* (3) 151–153

Goldschmidt E (1968) On the etiology of myopia An epidemiologic study *Acta ophthal (Kbh) 46 Suppl 98* 1–172

Hegmann J P, Mash A J & Spivey B E (1974) Genetic analysis of human visual parameters in populations with varying incidences of strabismus *Amer J hum Genet 26* 549–562

Holm S (1937) Les etats de la refraction oculaire chez les paleonegrides au Gabon, Afrique equatoriale française Étude de race pour éclairer la genese de la refraction *Acta ophthal (Kbh) 25 Suppl 13*

Hvams S W, Pokotilo E & Shkurko G (1977) Prevalence of refractive errors in adults over 40 A survey of 8102 eyes *Brit J Ophthal 61* 428–432

Kelly T S B, Chatfield C & Tustin G (19 5) Clinical assessment of the arrest of myopia *Brit J Ophthal 59* 529–535

Lawætz B (1949) Undersøgelser af grønlændernes øjensygdomme. *Beretninger vedrørende Grønlands styrelse No 2* 70– 8

Li C C (197) Separation of common environment and dominance effects with classic kinship correlation models *Social Biol 24* 259–266

Lindner K (1944) Über den Einfluss von Umwelt und Vererbung auf die Entstehung der Schulmyopie *Albrecht v Graefes Arch Ophthal 146* 336–376

Littauer J, Sagild U, Jespersen C S & Andersen S (1976) Height and weight in Greenlanders *Nord Counc arctic med Res Rep 14* 46–61

Mash A J, Hegman J P & Spivey B E (19 5) Genetic analysis of indices of corneal power and corneal astigmatism in human populations with varying incidences of strabismus *Invest Ophthal 14* 826–832

Morgan R W & Munro M (1973) Refractive problems in northern natives *Canad J Ophthal 8* 226–228

Morgan R W, Speakman J S & Grimshaw S E (1975) Inuit myopia An environmentally induced epidemic? *Canad med Ass J 112* 575–577

Oakley K H & Young F A (1975) Bifocal control of myopia. *Amer J Optom physiol Optics 52* 58–764

Otsuka J (1967) Research on the etiology and treatment of myopia *Acta Soc ophthal Jap 71 Suppl* 7–212

Post R H (1962) Population differences in vision acuity A review with speculative notes on selection relaxation. *Eugen Quart 9* 189–212

Sato T (1957) *The causes and prevention of acquired myopia* Yokohama.

Skeller E (1949) Øjensygdomme i Grønland. *Ugeskr Læg 111* 529–53

Skeller E (1954) Anthropological and ophthalmological studies on the Angmagssalik Eskimos *Meddelelser om Grønland 107 (4)* 1–211

Slataper F J (1950) Age norms of refraction and vision. *Arch Ophthal (Chicago) 43* 446–451

Sorsby A (1972) *Modern Ophthalmology* p 29 Vol 3 2nd ed. Butterworth London

Sorsby A, Sheridan M & Leary G A (1962) Refraction and its components in twins *Spec Rep Ser med Res Counc (Lond) No 303* 1–43

Sorsby A Leary G A & Fraser G R (1966) Family studies on ocular refraction and its components *J med Genet 3* 269–273

Spivey B E (1976) Quantitative genetics and clinical medicine *Trans Amer ophthal Soc 74* 661–707

Steiger A (1913) *Die Entstehung der sphärischen Refraktionen des menschlichen Auges* Karger Berlin

Tscherning M (1883) Studien uber die Ätiologie der Myopie *Albrecht v Grafes Arch Ophthal 29 (1)* 201–272

Young F A (1958) An estimate of the hereditary component of myopia *Amer J Optom 35* 337–345

Young F A (1977) The nature and control of myopia *J Amer Optom Ass 48* 451–457

Young F A Leary G A Baldwin W R West D C Box R A Harris E & Johnson C (1969) The transmission of refractive errors within Eskimo families *Amer J Optom 43* 676–685

Young F A & Leary G A (1972) The inheritance of ocular components *Amer J Optom 49* 546–555

Author's address

P H Alsbirk M D
Department of Ophthalmology
Rigshospitalet
DK 2100 Copenhagen Ø
Denmark

From the Medical Districts of South Greenland
and from the Eye Department Kommunehospitalet Copenhagen
(Head P Brændstrup)

PREVALENCE OF PINGUECULA IN GREENLAND AND IN COPENHAGEN AND ITS RELATION TO PTERYGIUM AND SPHEROID DEGENERATION

BY

M S NORN

The prevalence of pinguecula has been studied by slit lamp examination of 659 Eskimos in South Greenland and 810 Caucasians in Copenhagen.
The overall prevalence was found to be 56 per cent in Greenland against 41 per cent in Copenhagen ($P < 0.001$) and to rise with increasing age though with a fall after the age of 60 among Greenland women
Pingueculae (measured by their vertical height) are largest among Greenlanders and larger in males than in females They increase in size with increasing age
They are generally located nasally in Greenlanders and temporally in Copenhageners
The prevalence of pinguecula is almost 1⅓ times higher among Greenlanders than among Copenhageners while that of spheroid degeneration and that of pterygium are 3 times and slightly over 10 times higher respectively
The incidence of pinguecula and spheroid degeneration are correlated per site and per subject in the two geographically different series
Pterygium is not correlated with regard to site this being always located nasally Pterygium practically never harbours spheroid degeneration neither in its body nor in its head (81 pterygia)
Pinguecula and pterygium are therefore to be regarded as two different disorders while spheroid degeneration is related to pinguecula

Key words cornea – sclera – pinguecula – pterygium – degeneration, spheroid limbal corneoscleral – Greenland Eskimos – Copenhagen Caucasians – prevalence – correlation

Received February 6 1978

Pinguecula is a yellowish circumscribed slightly elevated pad like formation seen on the exposed part of the bulbar conjunctiva close to the cornea It consists of connective tissue subjected to elastoid degeneration

Many investigators believe pinguecula to be a precursor of pterygium

The object of the present study has been to estimate the prevalence of pinguecula within two geographic territories One is South Greenland where pterygium is a frequent disorder (8 6 per cent) and the other Copenhagen where pterygium is rare (0 7 per cent Norn 1978b)

The report comprises recording of the three paralimbal dgenerative processes (pterygium pinguecula and spheroid degeneration) all characterized histologically as elastoid degeneration and probably all due to climatic factors presumably ultraviolet light

Method and Material

All pingueculae of minimum 0 2 mm were recorded when disclosed by slit lamp examination with ten times magnification

The statistical calculation was based on Student s *t* test $P < 0\ 05$ is regarded as significant

The total series comprised 1469 subjects 659 Eskimos examined in South Greenland (arctic territory with much ultraviolet light) and 810 Caucasians in a big city (Copenhagen with a temperate moist climate) The prevalences of pterygium and spheroid degeneration as well as the age and sex incidences of the series under review have been described previously (Norn 1978a b)

Results

Pinguecula was more frequent in Greenland than in Copenhagen The overall prevalence per subject was 56 per cent among Greenland Eskimos against 11 per cent among Caucasians in Copenhagen ($P < 0\ 001$)

Fig 1 shows the age and sex curves The prevalence of pinguecula rises with increasing age until a level is reached after the age of 60 years However among female Greenlanders the prevalence declines after the age of 60 years ($P < 0\ 001$ in the age group of 40–59 compared with that of 60 or older)

A tendency was noticed towards preponderance of males but the difference was not significant

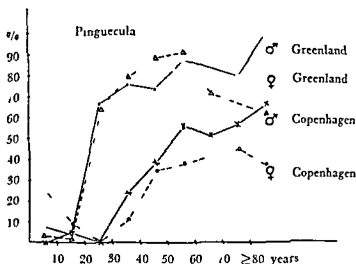


Fig 1

Prevalences of pinguecula among 659 Eskimos in South Greenland and 810 Caucasians in Copenhagen distributed according to sex and age

Abscissa 10 year age groups

Ordinate incidence of pinguecula

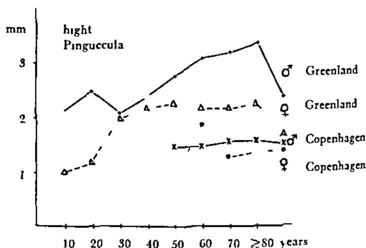


Fig 2

Size of pinguecula Dependence on age sex and geographic territory (Eskimos in South Greenland and Caucasians in Copenhagen)

Abscissa 10 year age groups

Ordinate Mean vertical height of pinguecula in millimetres

(The curves for young Copenhageners have been ruled out The groups are too small)

In 43 per cent of the Greenlanders and 54 per cent of the Copenhageners he *pinguecula* was typically *triangular* with its base towards the cornea and ts apex towards the inner or the outer canthus In 7 and 6 per cent respective y the shape was horizontal oblong and in the remaining cases more or less ircular

The vertical height of the pinguecula was noticed to rise with increasing age being the highest in the Greenlanders and particularly high in men (Fig 2)

Table I shows a significant difference with regard to sex between the Green anders and the Copenhageners The vertical height averaged 2 43 mm in the Greenlanders and 1 48 mm in the Copenhageners

Pingueculae above 3 mm in height were present in 21 8 per cent of the Greenlanders against 1 2 per cent of the Copenhageners ($P < 0\ 001$)

In Greenland relatively large pingueculae (above 3 mm) were most frequent een in elderly individuals (in 26 per cent aged over 50 years compared with 7 per cent of the younger individuals $P < 0\ 01$) In Copenhagen the age difference was not significant

In Greenland the mean height of the pinguecula was 2 83 mm in males and 20 mm in females In Copenhagen the corresponding figures were 1 61 and 40 mm

The nasally located pingueculae are generally larger than those located emporally In Greenland the average figures were 2 61 mm for the nasal and 11 for the temporal pigueculae In Copenhagen the figures were 1 79 and 92 mm respectively

ite Pingueculae have only been seen localized within the exposed part of the ulbar conjunctiva Among the Greenlanders the pinguecula was more de eloped and most often located nasally (82 47) and among the Copenhageners most often temporally (58 46) The differences are statistically significant Table II) In most cases it is located both temporally *and* nasally

Table I

Average vertical height of pinguecula Sex difference among Eskimos in Greenland and Caucasians in Copenhagen

	Men	Women	P
Greenland > 3 0 mm	30 %	17 %	≪ 0 001
Copenhagen > 2 0 mm	20 %	12 %	< 0 01

Table II

Sites of pinguecula in per cent of maximum possibilities. A total of [illegible] eyes with pinguecula ([illegible]72 Greenlanders with pinguecula = 5[illegible]2 [illegible] 2 = [illegible]44 temporal or nasal [illegible] [illegible]72 x 4 maximal sites – and 3[illegible]0 Copenhageners)

	Nasal	Temporal	*P*	Number of sites with pinguecula subject
Greenland	52 ([illegible]44)	4[illegible] (3[illegible]1[illegible]44)	< 0.001	[illegible]
Copenhagen	45 (303 66[illegible])	[illegible] ([illegible] 660)	< 0.01	209

Fig 3 illustrates the distribution over sites in the two series. In Greenland binasal and all four possible sites (two nasal and two temporal in the same individual) were the most frequent combinations. In Copenhagen bitemporal and monotemporal occurrence predominated. The Figure further includes [illegible]

Pinguecula %

Greenland	nasal		Copenhagen	Basel
13.6	O›	O	13.9	0
3.0	‹O	O	19.[illegible]	[illegible]
25.[illegible]	O›	‹O	9.9	4[illegible].6
3.5	‹O›	O	6.2	[illegible]
1.1	‹O	‹O	1.9	0.5
3.0	‹O	O›	1[illegible].9	1
12.[illegible]	‹O›	‹O	3.[illegible]	[illegible].3
[illegible]	‹O›	O›	11.7	1.[illegible]
25.[illegible]	‹O›	‹O›	15.1	4[illegible].3
100%			100%	100

Fig 3

Site of pinguecula among 6[illegible] Eskimos in Greenland and 510 Caucasians in Copenhagen (compared with a revision of Hinnen's series of 412 Caucasians from East Switzerland 1921). Whether the disorder has affected the right or the left eye has [illegible] been considered, but attention has been focused on the number of pinguecula [illegible] their distribution over nasal and temporal sites, a total of nine pos[illegible] combinations

Table III

Interrelation of pinguecula and pterygium in the same subject (659 Greenlanders and 810 Copenhageners) The χ^2 test for two independent samples

	Greenland		Copenhagen	
	+ pteryg	− pteryg	+ pteryg	− pteryg
+ pinguecula	15	32	5	325
− pinguecula	12	2 5	1	4 9

$\chi^2 = 11.48$ $P < 0.001$
$\chi^2 = 2.94$ $P > 0.05$ n s

the sake of comparison a calculation based on Hinnen s series from Basel in 1921

Pingueculae occur on an average at more sites in the eyes of Greenlanders than in those of Copenhageners

Relation to pterygium

Concurrence of pterygium and pinguecula at the same site was seen in no more than three cases (3/81 = 4 per cent)

Note however *that a pinguecula might be concealed to be concealed or have been modified in the "body" of the pterygium thus being non detectable*

In the Copenhagen series no correlation was found between pinguecula and pterygium, even if present in the same subject (in the same or the opposite eye) whereas a correlation was noticed in the Greenland series (Table III)

The pinguecula was no larger in the males with concurrent pterygium compared with males without pterygium (in the Greenland series the mean vertical pinguecula height was 2 82 mm in subjects with pterygium and 2 83 mm in the total series In the Copenhagen series the corresponding figures were 1 59 and 1 61 mm respectively) The pinguecula was perhaps a little larger in the female Greenlanders with pterygium (2 69 against 2 20 mm) whereas it was smaller in the female Copenhageners (0 73 against 1 40 mm)

Relation to spheroid degeneration

The incidences of spheroid degeneration and pinguecula were correlated in the two geographically distinct series both per subject and per site (all

$P < 0.001$ (Norn 1976a) the majority of the spheroid globules cannot be found on the pinguecula.

This means that the spheroid globules may be characterized as making a pinguecula a kind of spherical condensation of the elastoid degeneration of the pinguecula.

If a pterygium represented a further development of a pinguecula made in the body of the pterygium, we should expect to find a frequent occurrence of spheroid degeneration on a pterygium.

This was not so in our material. In fact, in no more than three of the [illegible] cases of pterygium was spheroid degeneration seen in relation to this disorder. In the stated three cases spheroid globules were present on the cornea close to the head of the pterygium. Only in one case spheroid degeneration was observed on the body of the pterygium (Norn 1976b).

Discussion

Skeller in 1949 examined 359 individuals in North and West Greenland and noticed pingueculae in 71 per cent of the males and 46 per cent of the females, i.e. prevalences corresponding to or being a little on the large side of the figures arrived at for South Greenland.

For boys under 10 years of age the percentage was 8 in Skeller's series compared with 7.1 in the present.

Like pterygium, pinguecula seems to be a very frequent disorder in Greenland (Clemmesen 1976). However, no further investigations have been published from Greenland.

Young & Finlay (1975) examined 929 individuals in Newfoundland and Labrador (Eskimos, Red Indians and Caucasians). They recorded prevalence and plotted age incidence curves on pinguecula corresponding approximately to the findings in the Greenland series under review. Note that the age incidence curve for females fell after the age of 70 years, while the incidence among males rose to nearly 100 per cent, all in conformity with the conditions in the present Greenland series.

Forsius & Eriksson (1963) examined 700 individuals with a binocular magnifying glass in Helsingfors, Finland. They likewise noticed preponderance of males and a rising prevalence with increasing age, though perhaps with a fall among women aged over 70 years.

In addition, they found a higher prevalence among outdoor workers than among indoor workers. The criteria employed do not permit a direct comparison with the series under review, but the prevalences seem to come close to

those found for Greenlanders (83–78 per cent among males and 78–50 per cent among females outdoor and indoor respectively)

In Basel Switzerland Hinnen in 1921 examined 462 individuals in Gullstrand s slit lamp He found the prevalence to rise with increasing age The overall prevalence was 38 per cent This finding corresponds approximately to the figures for the present Copenhagen series

The great difference in prevalence between the Greenlanders and the Copenhageners is significant the two series having been examined by the same person by the same procedure and within the same period

The difference between nasal and temporal location is remarkable Among the Greenlanders the pinguecula was most often located nasally and among the Copenhageners temporally

All previous investigators found preponderance of nasal site for pinguecula Young & Finlay (1975) in Canada found 611 nasal against 483 temporal Hinnen (1921) in Basel 16 per cent nasal against 2 per cent temporal and 20 per cent at both sites (i e a total of 38 per cent)

The temporal preponderance in Copenhagen is thus exceptional

Forsius & Eriksson (1962) on a rough grading of the size found the nasal pingueculae to be on an average larger than the temporal ones This view was borne out in the present investigation by direct measurement of the vertical height both in Greenland and in Copenhagen

Forsius (1972) likewise found the size of pinguecula to increase with increasing age However the curves perhaps suggest a minor decrease at extremely high ages Unfortunately the present Greenland series is too small to allow an estimate of a similar tendency in this series

Pterygium is believed by many workers to develop from a pinguecula (Fuchs 1891 Hilgers 1960 Cameron 1965) This view is supported by their histological structures both consist of hyaline connective tissue subjected to elastoid degeneration (Hogan & Zimmerman 1962 Hogan & Alvarado 1967 Klintworth 1972) and the raised prevalence within territories with much ultra violet light

Others hold that these disorders have a common causes but that development of pterygium requires an extra releasing factor (Garner 1972 Young & Finlay 1975)

Friede (1949) mentions episcleritis and peripheral defect of Bowman s membrane as additional causes of development of pterygium

Forsius & Eriksson (1962) found the pinguecula to be the largest in individuals with concurrent pterygium This observation could not be borne out in the present series

In 1963 Forsius & Eriksson declared that the incidence of pterygium among young individuals argues against its development from a pinguecula These workers have also seen pterygia develop without the presence of a pinguecula doubtless due to a genetic factor

The frequency of pterygium and band shaped climatic keratopathy are not correlated (Hawaii Finland – Forsius 1978)

I have previously demonstrated a correlation between spheroid degeneration and pinguecula (Norn 1978a) Simultaneous examinations of the three degenerative processes in two geographically distinct series with different prevalence of pterygium gave occasion for certain reflections

All three types of degeneration were found to be more frequent within the territory with much ultraviolet light and mainly outdoor work (South Greenland) than in Copenhagen

Pterygium was ten times more frequent (8 6 against 0 7 per cent, Norn 1979) spheroid degeneration three times more frequent (12 3 against 4 1 per cent Norn 1978a) and pinguecula scarcely one and a half times more frequent (56 against 41 per cent) in Greenland than in Copenhagen In other words an unequal distribution with a strikingly low incidence of pterygium among Copenhageners

The prevalences of the degenerative processes rise with increasing age though falls may be seen at very high ages both for pinguecula and spheroid degeneration but this is hardly the case with pterygium Pingueculae grow in size with increasing age but not so pterygia

The distribution between the nasal and temporal site is unequal In the series under review pterygium was always found nasally and never temporally Pinguecula and spheroid degeneration were most often located nasally in the Greenland series (nasal/temporal pinguecula 82 47 spheroid degeneration 84 20) but temporally in the Copenhagen series (temporal/nasal pinguecula 58 46 spheroid degeneration 37 32)

Pinguecula and spheroid degeneration tend to spread in approximately the same direction within the two geographically distinct territories whereas pterygium differs completely from these

Spheroid degeneration may occur in a pinguecula It may be characterized as a condensation of the elastoid degeneration of the connective tissue of the pinguecula.

If a pterygium represented a further development of a pinguecula we should expect to find larger pingueculae in individuals with pterygium and to observe spheroid degeneration in the body of the pterygium Such conditions were however not detectable by measuring the vertical heights of pingueculae not

-by close examination of pterygia in the slit lamp with ultraviolet and indirect white light

We may therefore be justified in regarding pterygium and pinguecula as two distinct disorders

Spheroid degeneration is related to pinguecula but not to pterygium

References

Cameron M E (1965) *Pterygium throughout the world* Charles C Thomas Publisher Springfield Ill

Clemmesen V (1976) Corneal diseases and scarring in Greenland a retrospective survey *Suid Afrikaanse Argief vir Oftalmologie* 57-62

Forsius H (1972) Climatic changes in the eyes of eskimos lapps and cheremisses *Acta ophthal (Kbh)* 50 532-538

Forsius H Pers commun (IV International symposium on circumpolar health)

Forsius H & Eriksson A (1962) Pterygium and its relation to arcus senilis pingvecula and other similar conditions *Acta ophthal (Kbh)* 40 402-410

Forsius H & Eriksson A (1963) Die Frequenz von Pingvecula and Pterygium bei Innen and Aussenarbeitern *Klin Mbl Augenheilk* 142 1021-1030

Friede R (1949) Die Pathogenese des echten Pterygiums *Acta ophthal (Kbh)* 27 507-515

Fuchs E (1891) Zur Anatomie der Pingvecula *Graefes Arch Ophthal* 37 143-191

Garner A (1972) Keratinod corneal degeneration *E E N T Monthly* 51 299-301

Hilgers J H (1960) Pterygium its incidence heredity and etiology *Amer J Ophthal* 50 635-643

Hinnen E (1971) Die Altersveranderungen des vorderen Bulbusabschnittes von 924 gesunden Augen, nach Untersuchungen am Spaltenlampenmikroskop *Z Augenheilk* 45 129-142

Hogan M J & Zimmerman L E (1962) *Ophthalmic Pathology* 2 ed p 253 Saunders Philadelphia

Hogan M J & Alvarado J (1967) Pterygium and pingvecula Electronmicroscopic study *Arch Ophthal (Chicago)* 78 1 4

Klintworth G K (1972) Chronic actinic keratopathy *Amer J Path* 67 327-342

Norn M S (1978a) Spheroid degeneration of cornea and conjunctiva. *Acta ophthal (Kbh)* 56 551-562

Norn M S (19 9) Pterygium Prevalens på Grønland og i København *Ugeskr Læg* 141 x (in Danish)

Skeller E (1949) Øjensygdomme i Grønland *Ugeskr Læg* 19 529 (in Danish)

Young J D H & Finlay R D (1975) Primary spheroidal degeneration of the cornea in Labrador and Northern Newfoundland *Amer J Ophthal* 79 129-134

Author's address

M S Norn M D
Dept of Ophthalmology
Københavns Kommunes
Hvidovre Hospital

Kettegård Alle 30
DK 2650 Hvidovre
Denmark

Department of Ophthalmology (Head Sven Erik G Nilsson)
University of Linkoping Sweden

THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

I The Basal and Intermediate Layers of the Perilimbal Zone

BY

STEFAN LATKOVIC and SVEN ERIK G NILSSON

The present paper on the perilimbal zone of the guinea pig conjunctival epithelium describes the ultrastructural organization of the basal cell layer including basal epithelial cells melanocytes and small lymphocytes as well as the intermediate cell layers consisting of polyhedral cells and processes from the melanocytes and the small lymphocytes Particular attention is paid to those cell features that might be associated with phagocytosis autophagy and transport mechanisms

Key words conjunctival epithelium – perilimbal zone – guinea pig – ultrastructure – transmission electron microscopy

In humans the most common eye diseases affect the conjunctiva The majority of these diseases are infectious and/or inflammatory in nature It may therefore be of general interest to study at the ultrastructural level the morphological changes as well as the mechanisms of defense of the conjunctival epithelium that occur in such diseases Bacteria of different strains of *Shigella* (Racz & Tenner 1963) *Salmonella* (Tenner et al 1971) and *Listeria* (Zimianski et al 1974) have been found inside the conjunctival epithelial cells In the case of *Listeria* where bacteria were observed intracellularly within membrane limited vacuoles the process was regarded as phagocytosis In connection with *Salmonella* (light microscopy) the terms endocytosis and pha-

Received June 8 1978

cytosis were used However the significance of the finding of intracellularly located bacteria in this as well as in other epithelia is still unclear and the question as to whether bacteria penetrate into the host cells or are phago cytized by them needs to be investigated further

In an electron microscopic investigation to be published (Latkovic & Nilsson 1979b) we demonstrated uptake of latex microspheres a nonmetabolic, non toxic marker by conjunctival epithelial cells indicating active phagocytic capability of these cells An additional study concerning bacteria is in pro gress

As a background for the investigations outlined above a knowledge of the architecture of the normal conjunctival epithelium and of the ultrastructure of its cells including the migratory cells was needed

The aims of the present series of papers are (1) to give a detailed de scription of the ultrastructural organization of all morphologically different zones of the normal conjunctival epithelium as studied in a continuous strip of tissue from the limbus to the lid margin which has not previously been done and (2) to emphasize those morphological features that might be associated with the processes of endocytosis autophagy and transport

Most of the recent and/or more complete ultrastructural investigations of the conjunctival epithelium concern the perilimbal zone (Wanko et al 1964 Hogan et al 1971 Radnot 1971) the palpebral zone (Takakusaki 1969) or the tarsal zone (Dark et al 1974 Greiner et al 1977) A short report on bulbar conjunctiva was included in a study of ocular pemphigus (Carroll & Kuwabara 1968) and a very short survey of bulbar fornical and tarsal conjunctiva was published by Weingeist (1973) In older studies in which specimens may have also been taken from the fornices the descriptions are generally less com prehensive and to a large extent concentrated upon certain cell organelles cell types or intercellular relations (Suzuki 1956 1957 de Toledo et al 1957 Shi buya 1958 Fujiyama 1961 Segawa 1962 de Toledo 1966)

Material and Methods

Four clinically healthy adult pigmented guinea pigs weighing 350–500 g were used for this study The animals were anaesthetized intraperitoneally with 30 mg/kg b w sodium pentobarbital (Nembutal® Abbott) and perfused (pressure 120 mmHg) via the left ventricle with 4% glutaraldehyde in 0 1 M sodium cacodylate buffer at pH 7 2 Each eye was excised in one piece including the eyelids Fixation was con tinued by immersion in glutaraldehyde solution as above but at +4 C during the next 3 h. 1–2 mm wide parallel sided strips of the conjunctiva were then cut, ex

tending from the corneal periphery to the margin of the lower eyelid Part of the abundant subconjunctival tissue was removed After a rinse in 0 1 M sodium cacodylate buffer the strips of conjunctival tissue were post fixed in 1 % osmium tetroxide in veronal acetate buffer at pH 7 2 for 1 h Acetone in increasing concentrations was used for dehydration

For light microscopy the tissue blocks were embedded in methacrylate cut in large sections at right angles to the limbus on a Porter Blum microtome and stained with toluidine blue The light micrographs were taken as a series of overlapping negatives at the same magnification The final micrograph represents a montage of exactly matched parts

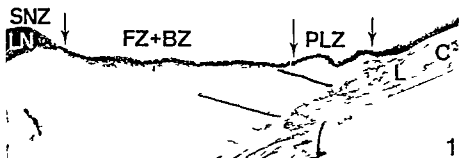
For transmission electron microscopy embedding was done in Vestopal W The sections were cut on the LKB Ultrotome parallel to the limbus The sections were first placed on Formvar filmed grids coated on the back with carbon and then stained with uranyl acetate for 1 h and lead citrate for 5 min The specimens were examined in a Philips EM 300 at an accelerating voltage of 80 kV (or in a few cases at 60 kV)

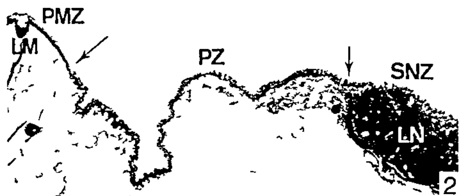
Observations

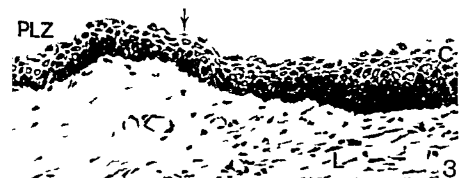
General aspects

The general appearance of the guinea pig conjunctiva is shown in low power light micrographs (Figs 1 and 2) It is composed of a stratified non keratinizing epithelium and an underlying stroma The main part of the epithelium is three to five layered consisting of basal intermediate (polyhedral) and superficial epithelial cells as well as specialized cells (melanocytes goblet cells) in certain zones and occasional migrating cells Towards the limbus and the lid margin the epithelium is more complex gradually changing into other types of epithelia (limbal corneal and epidermal) The stroma is composed of loose connective tissue blood and lymph vessels and a larger number of migrating cells than in the epithelium A lymphoid nodule adherent to the overlying epithelium is located at about 1/3 of the distance between the limbus and the lid margin

The conjunctival epithelium can be divided topographically into a number of zones (Figs 1 and 2) that will be described in four subsequent papers the perilimbal zone (PLZ) in the present paper I (the deeper layers) and in paper II (the superficial layer) (Latković & Nilsson 1979a) the bulbar zone (BZ) the zone of the fornix (FZ) and the supranodular zone (SNZ) (overlying the lymphoid nodule) in paper III (Latković 1979a) the palpebral zone (PZ) and the perimarginal zone (PMZ) in paper IV (Latković 1979b) The limbal epithelium is not included in the present study since it does not belong to the conjunctiva proper Except for the greater number of cell layers melanocytes and melanosomes (melanin granules) in the limbal epithelium no major differences between the perilimbal and the limbal epithelia were observed







Figs 1–3

Low power light micrograph of the perilimbal zone (PLZ) the bulbar zone (BZ) the zone of the fornix (FZ) and part of the supranodular zone (SNZ) of the conjunctival epithelium of the guinea pig C cornea L limbus LN part of the subepithelial lymphoid nodule × 60

2 Low power light micrograph of the supranodular (SNZ) palpebral (PZ) and perimarginal (PMZ) zones of the conjunctival epithelium LN lymphoid nodule LM lid margin × 60

3 Survey light micrograph of the epithelium of the perilimbal zone (PLZ) of the conjunctiva and of the epithelium overlying the limbus (L) The number of cell layers is greater in the latter than in the former epithelium The dark cells in the basal and suprabasal layers are rich in melanin pigment C cornea × 370



Fig 4

Survey electron micrograph from the perilimbal zone A layer of superficial cells (S) intermediate layers of polyhedral cells (P) and a layer of basal cells (B) are seen A melanocyte process (MP) extends into the suprabasal layer Small lymphocytes (LY) with rather electron lucent cytoplasm are found between the basal and suprabasal cells DP dendrite like processes from lymphocytes or melanocytes N nuclei M melanosomes CS conjunctival stroma Arrow points to the basal lamina x 4800

The basal cell layer of the perilimbal zone

The basal cell layer of the conjunctival epithelium was continuous with the same layer of the limbal and the corneal epithelium (Figs 1 and 3) This single cell layer was composed of basal epithelial cells a certain number of melanocytes and occasional small lymphocytes (Figs 4–7) The basal lamina separated from the epithelial cells by an electron lucent zone followed closely the inner border of the basal cells (Figs 4 5 and 7) The lamina was connected to the basal cells by half desmosomes (Fig 5)

The basal cell

The basal cell had a rather irregular shape (Figs 4 and 5) The nucleus was large and occupied the greatest part of the intracellular compartment Thick bundles of parallel microfilaments some of them surrounding the nucleus constituted a prominent feature of the cytoplasmic content (Figs 4–6) Rather small round mitochondria were enmeshed within the microfilament bundles (Fig 6) A Golgi complex was often seen in the supranuclear part of the cell Free ribosomes (Fig 6) and rough surfaced endoplasmic reticulum were found in the cell periphery A few vesicles were distributed randomly throughout the cytoplasm (Fig 5) Mature melanosomes were seen in most basal cells located both in the perinuclear zone and in the cell periphery (Figs 5 and 6) They were not membrane bound The cytoplasm projected numerous short and thin extensions loosely interdigitated with those of the adjacent cells (Fig 7) In the outer part of the basal layer adjacent cells w re connected by desmosomes (not shown in the figures)

The melanocyte

Melanocytes were interspersed irregularly between the basal cells They consisted of a rounded cell body and a number of dendrite like cytoplasmic processes (Fig 7) A large nucleus was surrounded by a thin layer of cytoplasm containing numerous melanosomes The cytoplasmic matrix was often slightly more electron lucent than that of the basal cells Microfilaments were absent both in the cell body and the dendrites Free ribosomes were scattered throughout the cytoplasm Otherwise the cell organelles were comparatively few The cell body as well as the dendrites showed a smooth cell membrane without junctional complexes of any kind and without interdigitations with the facing cells

The dendrites penetrated between the cells in the basal intermediate and sometimes superficial cell layers (Figs 4 and 7) The number of melanosomes


F
R
F
MI
M
6

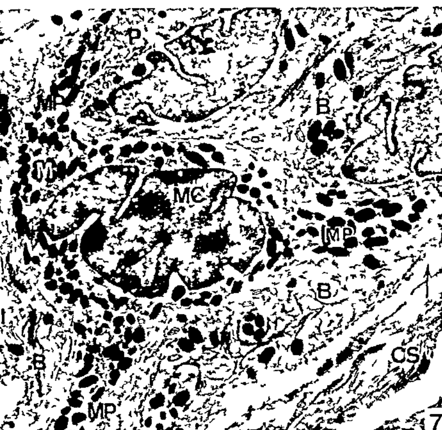


Fig 7

ɔcyte (MC) with dendrite like processes (MP) located between the basal (B) ıd polyhedral (P) cells Melanosomes (M) are numerous in the melanocyte but they ın be found in all adjacent cells Arrow points to the basal lamina CS conjunctival roma I loose interdigitations and rather wide intercellular space between two basal cells × 8700

Figs ɔ–6

Basal cell (B) with deeply crenated nucleus (N) bundles of microfilaments (F) and arse mitochondria (MI) VE vesicle LY lymphocyte M melanosomes Arrows ɔ nt to the basal lamina with half desmosomes × 16 000

Part of a basal cell with free ribosomes (R) melanosomes (M) and small mito ıondria (MI) enmeshed within thick bundles of microfilaments (F) × 33 600

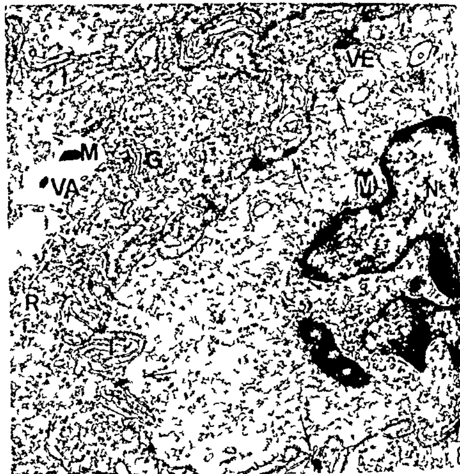


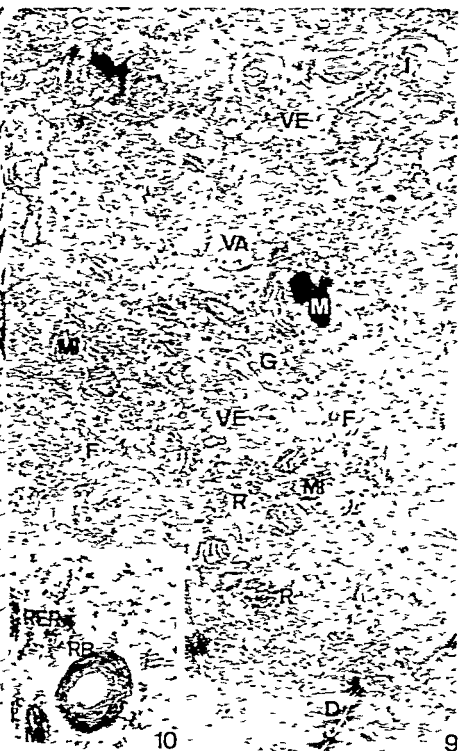
Fig 8

Two adjacent polyhedral cells (a dark one to the left and a light one to the right) in the intermediate layer Note the difference in the electron density of the cytoplasmic matrix The cell processes are interdigitating (I) with rather narrow and uniform intercellular spaces Vesicles (VE) and vacuoles (VA) are located predominantly in the supranuclear part of the cells The vesicles seem to be empty whereas the vacuoles contain granulated material membrane profiles and what appears to be melanin (M) G Golgi complex N nucleus R free ribosomes Arrows point to desmosomes ×21 500

Figs 9–10

9 The supranuclear portion of a polyhedral cell in the outer part of the intermediate layer The cell processes show tight interdigitations (I) In certain places the cells are connected by desmosomes (D) Vesicles (VE) are concentrated in the proximity of a moderate Golgi complex (G) and in the apical part of the cell Melanosomes (M) appear to be lying free in the cytoplasmic matrix F microfilaments MI mitochondria R free ribosomes VA vacuole ×23 100

10 Residual body (RB) with concentrically arranged membranes RER short profiles of rough surfaced endoplasmic reticulum MI mitochondrion ×33 000


VE
VA
M
M
G
VE
F
F
M
R
R
RER
RB
M
D
10
9

seemed to decrease along the dendrites. Towards the end, the dendrites contained only a very electron-lucent cytoplasmic matrix, and it was then impossible to differentiate them from other smooth, dendrite-like processes originating in lymphocytes.

The small lymphocyte

The small lymphocytes, found occasionally between the basal cells (Figs. [illegible] and 5), showed no interdigitations or intercellular junctions. The nuclear-cytoplasmic ratio was high, and the cytoplasm was low in electron density with very few intracellular organelles. The lymphocytes extended long dendritic processes (Fig. [illegible]) in a manner similar to melanocyte cell processes between the intermediate and superficial cells.

The intermediate cell layers of the perilimbal zone

In the major part of the perilimbal zone the intermediate layers were composed of two to three layers of polyhedral (or more or less flat) cells. Thus, including the basal and superficial cells, the epithelium of the perilimbal zone was four to five-layered. Lymphocyte cell bodies were sometimes found in the suprabasal cell layer, while the processes of lymphocytes and melanocytes could be seen also in the outer layers.

The polyhedral cell

The polyhedral cells were of varying sizes and shapes. They extended a large number of cytoplasmic processes, and their cell membranes were very [illegible] (Figs. 4, [illegible] and 9). In the suprabasal layer the intercellular spaces were [illegible], whereas in the outer layers (Figs. [illegible] and 9) the processes were [illegible] [illegible] and the cells joined by a larger number of desmosomes than in the [illegible] layers.

The cytoplasmic matrix of most polyhedral cells showed a rather high electron density ("dark cells") (Fig. 4). However, in a moderate number of randomly distributed polyhedral cells the cytoplasmic matrix was unusually electron-lucent and these cells therefore appeared as "light" cells (Figs. [illegible]). In all other respects both cell types seemed to be identical. The nucleus was large with sparse heterochromatin along the nuclear membrane (Figs. [illegible]). A moderate number of mitochondria, some profiles of rough-surfaced endoplasmic reticulum and a rather large number of free ribosomes were scattered throughout the cytoplasm. Microfilaments were only rarely arranged in [illegible] straight bundles. Most of them were grouped in loose arrangements dispersed in the cytoplasm (Fig. [illegible]

: The Golgi complex was well developed particularly in the cells of the outer layers and often located in the apical part of the cell (Fig 9) Vesicles of different sizes were seen in the vicinity of the Golgi complex Vesicles and vacuoles (the larger structures) were found in rather modest numbers in the inner cell layers whereas in the outer layers they were numerous and concentrated mainly in the apical cell compartment (Fig 9) Most vesicles had a clear appearance while many vacuoles were seen to contain finely or coarsely granulated material as well as membrane profiles (Figs 8 and 9) Melanosomes were found either free in the cytoplasm (Fig 9) or within vesicles or vacuoles as whole granules or parts of them (Fig 8) The latter arrangement was more frequently observed in the outer than in the inner cell layers

Primary lysosomes could not be positively identified What appeared to be secondary lysosomes (residual bodies) were seen in the outer layers however (Fig 10)

Discussion

A rather detailed description of the ultrastructure of the perilimbal zone of the human conjunctival epithelium was published by Wanko et al (1964) The authors also studied the same zone in the albino rat for comparison with the human material and found no significant differences Furthermore they pointed out the resemblance between the conjunctival epithelium and stratified squamous epithelia such as the oral mucosa and the combined stratum germinativum and stratum spinosum of the skin Hogan et al (1971) emphasized the many similarities between the corneal epithelium and the limbal zone of the conjunctival epithelium of the human eye

The general ultrastructural pattern of the perilimbal zone of the guinea pig conjunctival epithelium showed good conformity with that of the human counterparts as described in the two previously cited studies However certain dissimilarities were observed and will be pointed out

The most conspicuous difference was the absence of goblet cells Sections of tissue from different meridians of the perilimbal zone of the lower half of the eye were cut, both parallel with and perpendicular to the limbus but goblet cells were not seen In human preparations Wanko et al (1964) and Radnot (19 1) found an abundance of goblet cells in the perilimbal zone whereas Hogan et al (1971) described such cells in the periphery of the limbal zone According to Kessing (1968) the occurrence of goblet cells varies in different meridians and at different distances from the limbus It thus seems that differences as to location of the sections might be the main reason for the discrepancies

Another point of difference was the degree of pigmentation of the p[illegible] limbal zone In the pigmented guinea pig the pigmentation could be s[illegible] macroscopically and light as well as electron micrographs showed the p[illegible]sence of melanosomes in the majority of cells in this zone The conjunct[illegible] of the average white human contains less melanin (Wanko et al 1964 H[illegible] et al 1971)

Although melanin is synthesized in the melanocytes the melanosomes a[illegible] observed in almost all adjacent epithelial cells which implies some kind [illegible] transport across the cell membranes Birbeck et al (1956) suggested that t[illegible] melanosomes are phagocytized by the keratinocytes of the epidermis a the[illegible] accepted by numerous investigators Opinions differ however as to whe[illegible] the keratinocyte engulfs and pinches off a portion of a melanocyte dendr[illegible] containing melanosomes (Birbeck et al 1956 Cruickshank & Harcourt 1[illegible] Mottaz & Zelickson 1967 Prunieras 1969 Klaus 1969a Okazaki et al 1[illegible] or takes up melanosomes extruded into the intercellular space by the mela[illegible]cyte (Swift 1964) The phagocytic capability of the keratinocytes has also be[illegible] demonstrated by the uptake of metabolically inert markers such as carb[illegible] particles (Platt 1963) and latex microspheres (Wolff & Konrad 1972) [illegible] melanosomes are also found in the conjunctival epithelial cells adjacent to t[illegible] melanocytes it seems reasonable to expect an identical transport mech[illegible] i e phagocytosis by the basal and polyhedral epithelial cells

The presence of two kinds of polyhedral cells "dark" and light ones h[illegible] not previously been reported for the conjunctival epithelium However [illegible] micrograph of intermediate layers of human conjunctival epithelium pathol[illegible] but not commented upon by Carroll & Kuwabara (1968) showed polyhed[illegible] cells with different electron density of the cytoplasmic matrix Light and da[illegible] cells were also observed in the similar epithelium of pterygium (van der Zyp[illegible] et al 1975) and interpreted as reflecting the differences in metabolic activ[illegible] of the cells Dark and light cells were observed in various normal and p[illegible] logic animal tissues by many investigators and generally interpreted as ar[illegible] facts of immersion fixation (Palay et al 1962 Ganote & Moses 1968) T[illegible] interpretation was supported by the simultaneous findings of changes in m[illegible] cellular organelles characteristic of poor fixation However dark and li[illegible] cells were also found in the epithelium of the choroid plexus after perfu[illegible] fixation (Dohrman 1970) It was suggested that the dark and light cells m[illegible] represent varying stages of cellular hydration Our material showed no ev[illegible]dence of poor fixation

The finding of small lymphocytes in the cell layers of the perilimbal re[illegible] possibly including some immunologically competent cells suggests that t[illegible] conjunctival epithelium is capable of immune defense

The intercellular spaces were wider close to the basal lamina than farther ut in the epithelium This arrangement might imply that the intercellular route of transport is of significance basally in the epithelium whereas farther ut the intracellular route might be the more important one

As the cells moved from the basal layer and reached the intermediate layers he Golgi complex and the vesicles increased in size and number and were oncentrated in the apical part of the cell At this level some vacuoles were een to contain finely or coarsely granulated material membrane profiles ind/or melanosomes The vesicles observed in the vicinity of and most likely riginating from the Golgi complex probably contain products synthesized or ackaged there mucopolysaccharides hydrolytic enzymes etc (Rambourg 19/1 Rhodin 1974 Lloyd & Beck 1974) Primary lysosomes however cannot be dentified with certainty solely on the basis of their morphology Other vesicles ould be part of a transport mechanism across the cell membrane the smallest f them possibly being the pinocytotic vesicles The presence of a few multi esicular and residual bodies as well as the findings of granulated material nembrane profiles and also what appeared to be partly decomposed melano omes within many vacuoles can be interpreted as signs of autophagocytosis Hori et al 1968 Klaus 1969b Lloyd & Beck 19/4)

An abundance of filaments is found in cells with a need for a well developed ytoskeletal system (Fawcett 1966 Rhodin 19/4 Gipson 1977) Besides there s increasing evidence that the microfilaments are the contractile machinery of a non muscle cell (Wessells et al 1971) As such they participate in most forms f cell movement phenomena cytoplasmic streaming cell shape changes and ocomotion (e g cell sliding in connexion with corneal wound healing) and hagocytosis (Wessells et al 19/1 Bretscher & Raff 19/5 Burnside & Laties 1976 Gipson 1977 Gipson & Anderson 197/) Several of these qualities also eem to be relevant in connexion with the conjunctival epithelium and will be further discussed in a following paper (Latkovic & Nilsson 19/9a)

Acknowledgments

This investigation was supported by grants from the Swedish Medical Research Council (Project No 1?X 34) Rasmussen s Foundation the Research Committee of the Östergötlands Läns Landsting and Ollie and Elof Ericsson s Research Foundation

References

Birbeck M S C Mercer E H & Barnicot N A (1956) The structure and forma of pigment granules in human hair *Exp Cell Res 10* 505–514

Bretscher M S & Raff M C (1975) Mammalian plasma membranes *Nature (Lo 258* 43–49

Burnside B & Laties A M (1976) Actin filaments in apical projections of the prim pigmented epithelial cell *Invest Ophthal 15* 570–575

Carroll J M & Kuwabara T (1968) Ocular pemphigus *Arch Ophthal (Chicago)* 683–695

Cruickshank C N D & Harcourt S A (1964) Pigment donation in vitro *J Inv Derm 42* 183–184

Dark A J Durrant T E McGinty F & Shortland J R (1974) Tarsal conjunct of the upper eyelid *Amer J Ophthal 77* 555–564

De Toledo C A P (1966) Eletromicroscopia da melanina conjuntival lmb humana Cônsiderações sobre sua biossintese *Rev brasil Oftal 25* 305–310

De Toledo C Vallejo Freire A Oliveira Filho B & Souza Santos P (1957) Micro grafias eletronicas da conjuntiva ocular humana *Rev brasil Oftal 16* 219–231

Dohrmann G J (1970) Dark and light epithelial cells in the choroid plexus of mam mals *J Ultrastruct Res 32* 268–273

Fawcett D W (1966) *The Cell An Atlas of Fine Structure* pp 233–236 W B Saunders Philadelphia and London

Fujiyama H (1961) Electron microscopic studies on the conjunctiva sclera corn and lens crystallina *Acta Soc ophthal jap 65* 2101–2125

Ganote C E & Moses H L (1968) Light and dark cells as artifacts of liver fix tion *Lab Invest 18* 740–745

Gipson I K (1977) Cytoplasmic filaments their role in motility and cell shape *Inv Ophthal Visual Sci 16* 1081–1084

Gipson I K & Anderson R A (1977) Actin filaments in normal and migrating co neal epithelial cells *Invest Ophthal Visual Sci 16* 161–166

Greiner J V Covington H I & Allansmith M R (1977) Surface morphology of th human upper tarsal conjunctiva *Amer J Ophthal 83* 892–905

Hogan M J Alvarado J A & Weddell J E (1971) *Histology of the Human Ey* pp 65–111 128–136 W B Saunders Philadelphia London Toronto

Hori Y Toda K Pathak M A Clark Jr W H & Fitzpatrick T B (1968) A fine structure study of the human epidermal melanosome complex and its acid pho phatase activity *J Ultrastruct Res 25* 109–120

Kessing S V (1968) *Mucous gland system of the conjunctiva A quantitative norm anatomical study* pp 36–50 *Acta ophthal (Kbh) Suppl 95*

Klaus S N (1969a) Pigment transfer in mammalian epidermis *Arch Derm (Chica 100* 756–762

Klaus S N (1969b) Post transfer digestion of melanosome complexes and saltato movement of melanin granules within mammalian epidermal cells *J invest Der 53* 440–444

Latkovic S (1979a) The ultrastructure of the normal conjunctival epithelium of th guinea pig III The bulbar zone the zone of the fornix and the supranodul zone *Acta ophthal (Kbh)* Submitted for publication

Latkovic S (19 9b) The ultrastructure of the normal conjunctival epithelium of the guinea pig IV The palpebral and the perimarginal zones *Acta ophthal (Kbh)* Submitted for publication
Latkovic S & Nilsson S E G (1979a) The ultrastructure of the normal conjunctival epithelium of the guinea pig II The superficial layer of the perilimbal zone *Acta ophthal (Kbh)* Submitted for publication
Latkovic S & Nilsson S E G (19 9b) Phagocytosis of latex microspheres by epithelial cells of the conjunctiva In preparation for publication
Lloyd J B & Beck F (1974) Lysosomes In Beck F and Lloyd J B Eds *The Cell in Medical Science Vol I* Academic Press London New York
Mottaz J H & Zelickson A S (196) Melanin transfer a possible phagocytic process J invest Derm 49 605–610
Okazaki K Uzuka M Morikawa F Toda K & Seiji M (1976) Transfer mechanism of melanosomes in epidermal cell culture *J invest Derm 67* 541–547
Palay S L McGee Russell S M Gordon Jr S & Grillo M A (196) Fixation of neural tissues for electron microscopy by perfusion with solutions of osmium tetroxide *J Cell Biol 1*9 383–410
Platt H (1963) The engulfment of particulate and colloidal materials by epidermal cells *J Path Bact 86* 113–122
Prunieras M (1969) Interactions between keratinocytes and dendritic cells *J invest Derm 5*9 1–1
Racz P & Tenner K (1963) Die Bedeutung des intrazellularen Wachstums der pathogenen Mikroorganismen bei der Keratokonjunktivitis shigellosa und listeriosa *Verh dtsch Ges Path 47* 403–406
Radnot M (1971) Beitrag zur Feinstruktur der perilimbalen Zone der Bindehaut *Klin Mbl Augenheilk 159* 421–476
Rambourg A (1971) Morphological and histochemical aspects of glycoproteins at the surface of animal cells *Int Rev Cytol 31* 5 –114
Rhodin J A G (1974) *Histology* pp 34–38 44–4(Oxford University Press New York London Toronto
Segawa K (19( ) Epidemic keratoconjunctivitis light and electron microscopic study on the conjunctival epithelium *Jap J Ophthal 6* 143–154
Shibuya Y (1958) Electron microscopy by ultra thin specimens of normal human conjunctiva Report I Conjunctiva of the fornices *Acta Soc ophthal jap 6* 1?04–1?13
Suzuki A (19 6) Fine structure of normal human conjunctiva Electron microscopy in ultra thin section *Acta Soc ophthal jap 60* 441–459
Suzuki A (19 7) Fine structure of normal human conjunct va as revealed by electron microscopy in sections Report II *Acta Soc ophthal jap 61* 2?54–2258
Swift J A (19(4) Transfer of melanin granules from melanocytes to the cortical cells of human hair *Nature (Lond) 03* 976–97
Takakusaki I (1969) Fine structure of the human palpebral conjunctiva with special reference to the pathological changes in vernal conjunctivitis *Arch Histol jap 30* ?47–?82
Tenner C Racz P & Rédey B (19 1) Significance of the epithelial phase in experimental salmonella conjunctivitis *Acta Microbiol Acad Sci Hung 18* 16 –17
Wanko T Lloyd Jr B J & Matthews J (1964) The fine structure of human conjunctiva in the perilimbal zone *Invest Ophthal 3* ?85–301

Weingeist T A (1973) The conjunctiva *Int Ophthalmol Clin 13* 85–91
Wessells N K Spooner B S Ash J F Bradley M O Luduena M A Taylor E L Wrenn J T & Yamada K M (1971) Microfilaments in cellular and developmental processes *Science 171* 135–143
Wolff K & Konrad K (1972) Phagocytosis of latex beads by epidermal keratinocytes in vivo *J Ultrastruct Res 39* 262–280
Zimianski M C Dawson C R & Togni B (1974) Epithelial cell phagocytosis of *Listeria monocytogenes* in the conjunctiva *Invest Ophthal 13* 623–626
Zypen F van der Zypen E van der & Daicker B (1975) Zur Ultrastruktur des Pterygum I Epithelgewebe und Drusen des konjunktivalen Anteils *Albrecht v Graefes Arch klin Ophthal 193* 161–175

Authors address
Dr Stefan Latkovic and Prof Sven Erik G Nilsson
Department of Ophthalmology
University of Linkoping
S 581 85 Linkoping
Sweden

Department of Ophthalmology (Head Sven Erik G Nilsson)
University of Linkoping Sweden

THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

II The Superficial Layer of the Perilimbal Zone

BY

STEFAN LATKOVIC and SVEN ERIK G NILSSON

The ultrastructure of the superficial cell layer of the perilimbal zone of the guinea pig conjunctival epithelium as seen in transmission and scanning electron micrographs is described The major interest is focused on the apparatus of vesicles vacuoles and cysts and their possible significance in connexion with transport functions and autophagocytic processes The importance of the cell surface components and the role of the microfilaments in phagocytosis are also discussed

Key words conjunctival epithelium – perilimbal zone – guinea pig – ultrastructure – transmission electron microscopy – scanning electron microscopy

In the previous paper (Latkovic & Nilsson 1979a) the basal and intermediate cell layers of the perilimbal conjunctival epithelium of the guinea pig were examined The present paper concerns the ultrastructure of the superficial cell layer of the same zone as observed in transmission and scanning electron microscopy This series of papers also including the other zones of the conjunctival epithelium (Latkovic 1979a b) is intended as a basis for a study of phagocytosis in this epithelium now in progress (Latkovic & Nilsson 1979b) In this connexion the superficial cells are of particular importance

The superficial layer of the perilimbal zone of the conjunctival epithelium has previously been studied by Wanko et al (1964) Radnot (1971a) and Pfister (1975) A similar epithelium that of the limbal zone has been described by

Received June 8 1978

Hogan et al (1971) The surface morphology of the human palpebral tar conjunctiva was examined by Takakusaki (1969) Dark et al (1974) r Greiner et al (1977)

Materials and Methods

The eyes of four clinically healthy adult pigmented guinea pigs were perfused w 4 % glutaraldehyde for the present study Specimens of perilimbal conjunctival , thelium were prepared for transmission electron microscopy according to the meth described earlier (Latković & Nilsson 1979a)

For scanning electron microscopy the acetone dehydrated specimens were sub to critical point drying using liquid carbon dioxide as the transitional fluid T specimens were mounted on specimen holders with conducting silver paint a vacuum evaporator with carbon and gold and examined at an angle of 45 in a JEOL SM 1 scanning electron microscope

Observations

The superficial layer of the perilimbal zone of the conjunctival epithelium of the pigmented guinea pig consists of a single layer of polyhedral cells It continuous with the corresponding layer of the limbal and bulbar zones Th cell nucleus was large and irregular (Fig 1) The cells were rich in mit chondria most of them concentrated in the supranuclear part of the cell (Fig 1 The number of free ribosomes was moderate and rough surfaced endoplasm reticulum occurred only sparsely The microfilaments appeared to be fewer the superficial cells than in the deeper cells and they were dispersed in th cytoplasm However a condensation of microfilaments was found subjacent t the free surface which at low magnification was seen as a discontinuous ele tron dense band parallel to the plasma membrane (Figs 1 and 2) The cyt plasmic matrix was of moderate electron density (Fig 1) and all cells of th superficial layer were uniform in this respect

Fig 1

Part of a polyhedral cell in the superficial cell layer A multitude of vesicles (VE) a vacuoles (VA) is the most prominent feature of the cytoplasm The vacuoles cont granulated material membrane profiles and melanosomes (M) in what appears to different stages of decomposition An intracellular cyst (CY) is located near the surface Microvilli project from all sides into the cavity The dark discontinuous b under the microvillous surface represents aggregated filaments I interdigitating pr cesses from adjacent cells MI mitochondria MV microvilli N nucleus NO nucleolus R free ribosomes × 14 500


MV
MI
VE
CY
M
VA
R
VA
VA
NO
M
N
VA
1

Vesicles and vacuoles were prominent components of the apical part of the cytoplasm (Figs 1 and 2) Most vesicles were small and empty looking often surrounding a well developed Golgi complex or aggregated subjacent to the free surface of the cell (Fig 2) The vacuoles were single or clustered in the latter case often communicating (Fig 1) They contained granulated material membrane profiles small vesicles and melanosomes (Figs 1 and 2) Some dense structures that seem to represent residues of partly degradated melanosomes were also found in the vacuoles (Fig 1) Multivesicular bodies were occasionally observed in the apical part of the cell (Fig 4)

Cytoplasmic processes of adjacent cells interdigitated tightly (Fig 1) and the intercellular space was narrow The cell membranes were connected by desmosomes Immediately below the free surface of the epithelium cells were bound together by tripartite junctional complexes (zonula occludens/tight junction zonula adherens and macula adherens/desmosome) which closed off the intercellular space partly seen in Fig 2

A large number of microvilli projected from the free surface of all cells (Figs 1–3 5–11) That which at low magnification appeared as a thin uneven coat on the surface of the microvilli (Fig 3) was seen at high magnification (Fig 11) as irregular filaments protruding from the tips and sides of the microvilli In other respects the plasma membrane of the microvilli did not differ from that of the rest of the cell Straight microfilaments ran axially through the centre of the microvilli and extended into the cytoplasm of the cell body (Fig 3)

Scanning electron microscopy of the free surface of the epithelium showed that the cells generally had an irregular pentagonal or hexagonal shape (Fig 5) The cell borders were somewhat elevated above the cell surface (Figs 5 and 6) The number and length of microvilli varied somewhat from cell to cell (Fig 5)

Figs 2–4

2 Supranuclear part of a superficial cell Small empty looking vesicles (VE) are aggregated subjacent to the free surface and also around the Golgi complex (G) Vacuoles (VA) contain granulated material and melanosomes (M) F *microfilaments* MV microvilli N nucleus R free ribosomes RER rough surfaced endoplasmic reticulum JC junctional complex × 28 100

3 Microvilli In certain places an uneven coat (arrows) can be seen on the surface of the microvilli Straight microfilaments (F) run axially through the microvilli A delicate network of microfilaments can be seen subjacent to the cell membrane × 68 500

4 A multivesicular body surrounded by mitochondria × 56 500

Some of the cells demonstrated small areas often slightly elevated where the microvilli were shorter and fewer (Fig 5) or sometimes absent (Fig 6) Such an area was invariably located near the cell border usually where three cells came together (Figs 5 and 6) Certain cells showed an opening in the area







Fig 5

Scanning electron micrograph of epithelial surface The cell borders (CB) are elevated *and the cells have an irregular shape* Depending upon the variance in density and *length of the microvilli* the cells have a lighter or darker appearance Arrows point *to areas where the microvilli* are fewer and shorter Slightly darker stripes run diagonally *across the* micrograph represent shadows caused by the folds in the *specimen CY* unroofed intracellular cyst ×6500

Figs 6–8

Intracellular cysts *in three stages* The bare elevated roof of a cyst is seen near the *cell border* (CB) *(Fig 6)* Through an opening in the roof the bottom of a cyst *covered with microvilli* can be observed (Fig 7) What seems to be the remains of the *roof of a cyst* are demonstrated in Fig 8 The bottom of the cyst is almost at the level of the cell surface

Fig 6 ×25 100 Fig 7 8 500 Fig 8 ×26 000

6



8


JC
CY
MV
9


CY
MV
JC
10


11

ading to an intracellular cyst the bottom of which was covered with short icrovilli (Fig 7) What seems to be the remnants of the membranous roof such a cyst are demonstrated in Fig 8

In transmission electron micrographs cysts of this kind were found only in e cells of the superficial layer located in the proximity of the free surface id near the cell border (Figs 1 9 and 10) They were identified by a number microvillous extensions from the walls of the cavity (Fig 1) In some sec ons an opening through the roof of the cyst was seen (Figs 9 and 10) The vity of the cyst appeared empty in all sections where an opening to the free rface was present (Figs 9 and 10) However in sections where no opening as found granulated or strand like material and membrane profiles were ten seen in the cysts (Fig 1) i e the same kind of material that was charac ristic of the vacuoles described above In a few cases what appeared to be the mains of melanosomes were also observed in the cavity

Cells containing the cysts did not show any differences in size or intra llular content as compared to cells without cysts

Discussion

omparisons of the superficial epithelial cells of the guinea pig conjunctiva ith the human counterparts as described by Wanko et al (1964) and Radnot 971a) for the perilimbal zone and by Takakusaki (1969) Dark et al (1974) id Greiner et al (1977) for the palpebral/tarsal zone showed good general onformity The dissimilarities concerned only the number and distribution of me intracellular organelles (mitochondria microfilaments vesicles vacuoles id melanosomes) and the goblet cells which were absent in the guinea pig he last two details were discussed in the preceeding paper (Latkovic & Nilsson 979a)

Figs 9–11

Intracellular cyst (CY) with an opening in the thin roof Inside the cyst micro lli (MV) project only *from its bottom The roof is lacking microvilli on both sides* he cyst is located near the cell border with a junctional complex (JC) I inter igitating processes between adjacent cells × 16 300

) Shallow almost unroofed intracellular cyst (CY) near a junctional complex (JC) he bottom is almost at the level of the cell surface and covered with rather sparse ort microvilli (MV) × 12 500

l High power electron micrograph showing filamentous structures protruding from ie tips of microvilli (obliquely cut) of a conjunctival epithelial cell × 16? 000

The microvillous surface of the guinea pig conjunctival epithelium was seen at high magnification to be covered by filaments projecting from the membrane Similar filaments have been observed on a number of diffe cells They are composed mainly of polysaccharides and considered as an in gral part of the cell membrane These filamentous structures occur only on free surface of a cell which can react with agents in the external envir ment They play a role in recognition contact and adhesion phenomena of cell surface e g in connexion with phagocytosis The filaments represent variation of the glycocalyx covering all cell surfaces For extensive revie of this voluminous subject see Martinez Palomo (1970) Winzler (1970) R bourg (1971) and Cook & Stoddart (1973) According to Parsons & Subje (1972) however distinction must be made between the cells that derive th thick coat of free polysaccharides from present mucus secreting cells and o cells of more general type with thinner coats A coat of acid polysacchari on the surface of the human conjunctiva was demonstrated by Radnot (19

The microprojections also greatly increase the actual surface of the e thelium which according to Harding et al (1974) should augment the proc of diffusion and active transport and also enhance the total activity of enzy associated with this region of the plasma membrane (Boyd & Parsons 196

The apical part of the polyhedral cells in the superficial layer was rem ably rich in vesicles and vacuoles These have been observed by a numb authors (Wanko et al 1964 Takakusaki 1969 Hogan et al 1971 Dark et 1974) but no proposals as to their function have been offered It is sugges that the primary lysosomes originate in association with the ribosomes and t Golgi complex (Lloyd & Beck 1974) Some of the membrane bound vesic ob erved in the vicinity of the latter therefore could be primary lysosomes is not possible to identify lysosomes solely on the basis of morphology without a positive reaction for acid hydrolases However the presence of s of the secondary phagosomes (autophagic vacuoles residual and multives bodies) could serve as indirect evidence for the occurrence of lysosomes in t superficial epithelial cells

Most vacuoles particularly the large ones had a content suggesting au phagy i e granulated material membrane profiles and melanosomes s of which appeared to be in different stages of degradation Many of the int cellular cysts in sections where an opening was not observed contained ma rial at least partly of the same kind as that of the vacuoles whereas cys which an opening was demonstrated seemed to be empty The interpreta of the whole procedure could be that the vesicles supply necessary enzy whereas vacuoles and intracellular cysts might be sequential steps in an a phagocytic process where cytoplasmic organelles are enclosed and diges

id which ends with the opening unroofing and emptying of a cyst This ay also be seen as an expression of ageing of the cells A participation of the sts in other functions cannot however be fully excluded

Intracellular cysts (or crypts) with an opening were observed in scanning ectron micrographs of corneal and conjunctival epithelium of the rabbit fister 1973 1975 Schmidt Martens et al 1976) and of tarsal conjunctival ithelium of the human (Greiner et al 197/) The latter authors interpreted e open crypts as collapsed and empty goblet cells In our material this uld *not be the case since goblet cells were not found in the perilimbal zone* fister suggests that the cysts initiate the desquamation of the cells thus ex osing the second cell layer an interpretation accepted also by Schmidt Mar ns et al (19/6) Our transmission electron micrographs do not support this eory So far we have not observed any full thickness holes the depth of the sts representing only a fraction of the total cell thickness and we have early shown that the microvilli are present on the walls of the cysts without sposing the second cell layer

The increasing number of vesicles and vacuoles observed as the cells move om the basal layer towards the surface of the epithelium and their concen ation in the apical part of the cells further support the presence of an active ansport system directed outwards On the other hand a clustering of small esicles subjacent to the free surface also suggests a participation of vesicles 1 a transport inwards across the plasma membrane including pinocytosis

In the superficial epithelial cells the microfilaments appear (1) as loose micro laments randomly distributed in the cytoplasm (2) as a thin zone of aggre ated microfilaments subjacent to the free surface of the cell and (3) as core ucrofilaments running axially through the microvilli Microfilaments are ound in almost every cell and are regarded as the contractile machinery of onmuscle cells (Wessells et al 19/1) The arrangements of the microfilaments nderlying the free surface of the epithelial cells of the guinea pig conjunc va resembles that of the terminal web filaments of the intestinal epithelium here an active movement of microvilli through interaction of core filaments nd terminal web filaments was demonstrated (Rodewald et al 19/6) Parti ipation of *subplasmalemmal filaments in the membrane movements in phago* ytosis has also been demonstrated for macrophages (Allison et al 19/1 Reaven Axline 19/3 Axline & Reaven 19/4) and for the retinal pigment epithelium Burnside & Laties 19/6) A similar aggregation of the microfilaments was bserved in the superficial cells of the corneal epithelium where they parti ipate in cell movements during wound healing (Gipson & Anderson 19/7) seems likely that the same mechanisms are also applicable to the conjunc val epithelium

Acknowledgments

This investigation was supported by grants from the Swedish Medical Res Council (Project No 12X 734) Rasmussen s Foundation the Research Committ the Ostergotlands Lans Landsting and Ollie and Elof Ericsson s Research Found.

References

Allison A C Davies P & Petris S de (1971) Role of contractile microfilaments macrophage movement and endocytosis *Nature (New Biol) 232* 153–155

Axline S G & Reaven E P (1974) Inhibition of phagocytosis and plasma membr mobility of the cultivated macrophage by cytochalasin B *J Cell Biol 62* 64–8

Boyd C A R & Parsons D S (1969) The fine structure of the microvilli of isola brush borders of intestinal epithelial cels *J Cell Biol 41* 646–651

Burnside B & Laties A M (1976) Actin filaments in apical projections of the pre pigmented epithelial cell *Invest Ophthal 15* 570–575

Cook G M W & Stoddart R W (1973) *Surface Carbohydrates of the Eukaryotic C* Academic Press London and New York

Dark A J Durrant T E McGinty F & Shortland J R (1974) Tarsal conjunc of the upper eyelid *Amer J Ophthal 77* 555–564

Gipson I K & Anderson R A (1977) Actin filaments in normal and migrating c neal epithelial cells *Invest Ophthal Visual Sci 16* 161–166

Greiner J V Covington H I & Allansmith M R (1977) Surface morphology of human upper tarsal conjunctiva *Amer J Ophthal 83* 892–905

Harding C V Bagchi M Weinsieder A & Peters V (1974) A comparative studi corneal epithelial cell surfaces utiizing the scanning electron microscope *Inv Ophthal 13* 906–912

Hogan M J Alvarado J A & Weddell J E (1971) *Histology of the Human E* pp 128–136 W B Saunders Philadelphia London Toronto

Latkovic S (1979a) The ultrastructure of the normal conjunctival epithelium of t guinea pig III The bulbar zone the zone of the fornix and the supraorbi zone *Acta ophthal (Kbh)* Submitted for publication

Latkovic S (1979b) The ultrastructure of the normal conjunctival epithelium of guinea pig IV The palpebral and the perimarginal zones In preparation f publication

Latkovic S & Nilsson S E G (1979a) The ultrastructure of the normal conjuncti epithelium of the guinea pig I The basal and intermediate layers of the p limbal zone *Acta ophthal (Kbh) 57* 106–122

Latkovic S & Nilsson S E G (1979b) Phagocytosis of latex microspheres by e thelial cells of the conjunctiva In preparation for publication

Lloyd J B & Beck F (1974) Lysosomes In Beck F and Lloyd J B Eds *The L in Medical Science Vol 1* Academic Press London New York

Martinez Palomo A (1970) The surface coats of animal cells *Int Rev Cytol* 29–75

Parsons D F & Subjeck J R (1972) The morphology of the polysaccharide coat mammalian cels *Biochim biophys Acta 265* 85–113

fister R R (1973) The normal surface of corneal epithelium a scanning electron microscopic study Invest Ophthal 12 654-668
fister R R (1975) The normal surface of conjunctiva epithelium A scanning electron microscopic study Invest Ophthal 14 267-279
adnot M (1971a) Beitrag zur Feinstruktur der perilimbalen Zone der Bindehaut Klin Mbl Augenheilk 159 421-426
adnot M (1971b) Les propriétés ultrastructurales de la surface de la conjonctive bulbaire Arch Ophtal (Paris) 31 09-716
ambourg A (1971) Morphological and histochemical aspects of glycoproteins at the surface of animal cells Int Rev Cytol 31 57-114
Reaven E P & Axline S G (1973) Subplasmalemmal microfilaments and microtubules in resting and phagocytizing cultivated macrophages J Cell Biol 59 12-27
Rodewald R Newman S B & Karnovsky M J (1976) Contraction of isolated brush borders from the intestinal epithelium J Cell Biol 70 541-554
chmidt Martens F W Busch L & Klaas D (1976) Fruhe Veranderungen der Conjunctiva nach Alkali Veratzung Albrecht von Graefes Arch klin Ophthal 201 135-147
akakusaki I (1969) Fine structure of the human palpebral conjunctiva with special reference to the pathological changes in vernal conjunctivitis Arch Histol jap 30 247-282
Vanko T Lloyd Jr B J & Matthews J (1964) The fine structure of human conjunctiva in the perilimbal zone Invest Ophthal 3 285-301
Wessells N K Spooner B S Ash J F Bradley M O Luduena M A Taylor E L Wrenn J T & Yamada K M (1971) Microfilaments in cellular and developmental processes Science 171 135-143
Winzler R J (1970) Carbohydrates in cell surfaces Int Rev Cytol 29 77-125

Authors address
Dr Stefan Latkovic and Prof Sven Erik G Nilsson,
Department of Ophthalmology
University of Linkoping
S 581 85 Linkoping
Sweden

The Eye Department (Head P E J Pohjanpelto)
Central Hospital of Paijat Hame
Lahti Finland

PULSE INDUCED INTRAOCULAR PRESSURE VARIATION AND RETROBULBAR ANAESTHESIA WITH AND WITHOUT ADRENALINE

BY

PEKKA POHJANPELTO

The intraocular pressure (IOP) and pulse induced IOP variation (IOPV) were registered before and five min after retrobulbar anaesthesia (RBA) in 34 operations The every second operation the anaesthetic (bupivacaine or lidocaine) was supplemented with adrenaline (epinephrine) Both IOP and IOPV were significantly lowered compared with the unanaesthetised fellow eye The change in IOP was 16 8 % with adrenaline and 15 1 % without it IOPV decreased 50 6 % with adrenaline and 36 9 % without adrenaline It is concluded that RBA reduces the intraocular blood supply and this is probably one reason for the IOP lowering influence of RBA Although to a smaller degree this vascular effect is also achieved without adrenaline

Key words adrenaline – epinephrine – retrobulbar anaesthesia – ocular surgery – intraocular pressure – intraocular blood supply

Adrenaline (epinephrine) added to the anaesthetic is known to cause va constriction and to decrease local blood circulation though the reaction m differ in different tissues (Dhuner & Lewis 1966) Reduction of the intraocu blood volume might be beneficial in many eye operations It would lower traocular pressure (IOP) and leave more space for the vitreous Vasoconstrict in the choroid might lessen the risk of expulsive haemorrhage during the op ation and the danger of haemorrhage when a perforation is performed in op ations for detachment of the retina might also be smaller

Received May 9 1978

On the other hand excessive vasoconstriction might cause detrimental ischaemia in the eye Caution has been advised in the use of adrenaline because of the danger of gangrene from local blocks in extremities such as fingers and toes (Moore 1955) Very rarely a retro ocular injection of adrenaline may induce spasm of the central retinal artery and lead to optic nerve atrophy Stallard 1973)

After retrobulbar injection of either lidocaine or adrenaline a 40–50 % decrease in corneal indentation pulse amplitudes have been demonstrated by use of dynamic tonometry (Syrdalen & Hørven 1970) The simultaneous injection of both drugs augmented the reduction significantly The purpose of the present study was to elucidate the potential effect on intraocular blood circulation of retrobulbar anaestesia (RBA) and adrenaline added to the anaesthetic by recording pulse induced IOP variation (IOPV) before and after RBA with Alcon Applanation Pneumatonograph The potential change in IOP was studied at the same time

Material and Methods

The material consisted of 26 patients whose mean age was 62 0 years range 24 to 81 These patients underwent a total of 34 operations in 32 eyes at which the measurements were made There were 12 iridectomies nine cataract extractions nine photocoagulations one retinal detachment operation one discission one cyclodialysis and one trabeculectomy Six patients have been operated on and examined more than once Iridectomy was performed on two patients and a cataract extraction on one patient was also performed in the fellow eye on a different occasion Two patients underwent photocoagulation three times and one patient twice

The anaesthetic agent was 0 5 % bupivacaine (Marcain Astra) in 12 operations and 0 5 % bupivacaine + adrenaline 5 μg/ml (Marcain Adrenaline Astra) in an equal number of operations 2 % lidocaine (Lidocain Orion) was the anaesthetic agent on five occasions and 2 % lidocaine + adrenaline 5 μg/ml Lidocain Adrenalin Orion) was used five times The volume of anaesthetic injected was 2 ml

Hyaluronidase (Hyason 150 UI/10 ml of anaesthetic) was added to the anaesthetic in nine operations As hyaluronidase did not seem to affect the results it was disregarded in analysing the results

The photocoagulations were performed without pre medication The other patients were given the routine pre medication in use at our hospital pethidine chloride 1 mg/kg and generally atropine 1 mg/10 kg

The anaesthetic was injected through the lower lid from the border of the lateral third over the lower rim of the orbit by directing a 30 mm long disposable needle inwards and upwards as deep as it would go 1 5 ml of anaesthetic was injected at this site The remaining 0 5 ml was injected while withdrawing the needle a few millimetres

The anaesthetic was chosen at random Adrenaline was used with the anaesthetic in every second operation and every other anaesthesia was performed without it

The unanaesthetised eye served as the control The examinations were made with an Alcon Applanation Pneumatonograph that enables the recording of IOPV four times magnified IOP and IOPV were measured in both eyes immediately before anaesthesia and 5 min after it The measurement was made first on the eye to be operated on In the first eight operations the measurements were made 2 5 and 10 min after anaesthesia but as the results were similar in all these measurements only the results obtained at 5 min were taken into consideration From 1 to 2 drops of oxibuprocaine was used for surface anaesthesia

The statistical analysis was made with sign test

Results

The mean IOP and IOPV values before RBA and five min after it are shown in Table I There was no difference in the effect on the results between bupivacaine and lidocaine and they were therefore grooped together in the statistical analysis

The high IOP values are attributable to the higher readings with the applanation pneumatonograph in the supine position than those given by Schiøtz tonometer for the same eye There were also some hypertensive eyes

The fall in IOP *in the anaesthetised eye is highly* significant ($P < 0.001$) statistically compared with the control eye which also showed a slight drop ($P < 0.01$) The reason for the reduction of IOP in the control eye is not clear It might be due to a central mediated sympathetic reaction However although the pneumatonograph has not a tonographic effect (Langham 1974) the role of repeated tonometry can not be excluded The avarage decrease in IOP of the anaesthetised eye was 16 8 ± 11 2 % when adrenaline was added and 15 1 ± 9 4 % without it The difference between these two groups is not statistically significant

The IOPV also decreased ($P < 0.001$) compared with the control eye in which it too fell slightly ($P < 0.01$) An example of a high response is pre-

Table I

Mean values of intraocular pressure (IOP mmHg) and pulse induced intraocular pressure variation (IOPV mmHg) before and five min after retrobulbar anaesthesia (RBA)

Anaesthetic solution	No of operations	Test eye				Control eye			
		IOP		IOPV		IOP		IOPV	
		Before RBA	After RBA	Before RBA	After RBA	Before RBA	After RBA	Before RBA	After RBA
0 5 % bupivacaine	[illegible]	24 8 ± 4 5	20 8 ± 3 6	2 6 ± 1 3	1 [illegible] ± 0 5	24 5 ± 4 3	23 1 ± 3 4	2 5 ± 0 9	2 4 ± 1 0
0 5 % bupivacaine + adrenaline	12	6 7 ± 3 2	22 0 ± 3 1	2 6 ± 0 9	1 2 ± 0 [illegible]	24 0 ± 1 7	22 8 ± 2 6	2 4 ± 0 7	2 2 ± 0 8
2 % lidocaine	5	21 8 ± 2 3	19 2 ± 1 3	2 0 ± 0 9	1 1 ± 0 4	22 8 ± 3 6	20 ± 3 3	2 2 ± 0 [illegible]	1 9 ± 0 7
2 % lidocaine + adrenaline	5	24 6 ± 4 7	21 0 ± 3 3	1 3 ± 0 1	0 9 ± 0 2	22 4 ± 3 0	21 2 ± 2 6	1 8 ± 0 1	1 7 ± 0 4

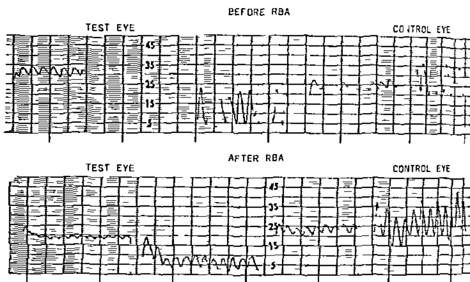


Fig 1
Records of IOP and IOPV before and five min after RBA with 0 5 % Marcain Adrenalin *This 60 year old female patient had the reduction of IOP from* 3? mmHg to 19 mmHg IOPV was lowered from 4 0 mmHg to 1 ?5 mmH

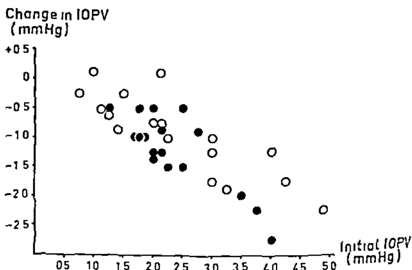


Fig 2
Change in IOPV five min after RBA (with adrenaline ● without adrenaline ○)

Table II

Mean values of pulse volume before and five min after retrobulbar anaesthesia (RBA)

Anaesthetic solution	No of operations	Pulse volume (μl) of the test eye		Pulse volume (μl) of the control eye	
		Before RBA	After RBA	Before RBA	After RBA
0 5 % bupivacaine	12	3 4 ± 1 5	2 5 ± 0 9	3 5 ± 1 0	3 5 ± 1 1
0.5 % bupivacaine + adrenaline	12	3 5 ± 0 9	2 0 ± 0 6	3 3 ± 0 9	3 2 ± 0 9
2 % lidocaine	5	3 0 ± 1 1	1 8 ± 0 6	3 0 ± 0 9	2 9 ± 0 8
2 % lidocaine adrenaline	5	2 6 ± 0 4	1 3 ± 0 3	2 7 ± 0 8	2 7 ± 0 8

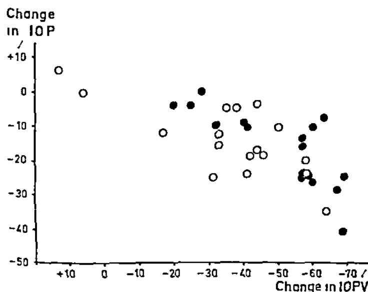


Fig 3

Effect of RBA on IOP and IOPV The change five min after RBA has been expressed in percentages (with adrenaline ● without adrenaline o)

sented in Fig 1 The decrease in IOPV was more evident with adrenaline th without it The average drop in IOPV when adrenaline was given was 5 ± 16 1 % and without it 36 2 ± 20 6 % (Fig 2) However the difference not statistically significant which may be due to the smallness of the mater

In assessing the results it must be borne in mind that when IOP falls th same pulse volume causes a smaller IOPV (Bynke 1968) However the dr in IOP established in the study could not cause such a great change in IOP in the anaesthetised eye thus it was a real decrease in pulse volume that w at issue The slight decrease in IOPV in the control eye was due to the dr in IOP and did not signify a change in pulse volume The approximate valu of the pulse volumes were calculated from the mean relationship between IOP and the intraocular volume changes (Langham 1974) The mean results a presented in Table II

There was some correlation between the changes in IOP and IOPV (Fig 3 The drop in IOP was associated with a distinct decrease in IOPV The drop in IOP was ≥ 20 % 12 times IOPV decreased by over 50 % in 10 of them reduction of IOPV occurred in two cases and neither of them showed a de crease in IOP In some eyes however even a great change in IOPV was as sociated with only a slight drop in IOP

The material was divided into three groups according to the change in pres sure and the average pulse volumes were calculated (Langham 1974) The results are presented in Table III The mean reduction of pulse volume w greater when IOP fell more and it seems that IOP would have declined mor readily if the pulse volume was great to begin with

Table III

Intraocular pressure change and mean values of pulse volume before and five min af retrobulbar anaesthesia (RBA)

Group	No of operations	Original mean IOP (mmHg)	Pulse volume (µl)	
			Before RBA	After RBA
I	11	24 3	2 8 ± 0 5	2 0 ± 0 6
II	12	24 8	3 3 ± 1 4	2 1 ± 0 9
III	11	25 7	3 7 ± 1 4	2 1 ± 1 0

Group I decrease in IOP ≤ 10 % group II 11–20 % and group III > 20 %

Discussion

BA had a lowering effect in this study on IOP and IOPV both in the pre nce of adrenaline and without it The results conform the earlier observations f Syrdalen and Hørven (1970) The lowering of IOPV was obviously attribu able to the decrease in the intraocular pulse volume which probably implies hat the intraocular blood supply diminished in conjunction with RBA Lido aine and bupivacaine did not have a decreasing effect on the circulation when njected subcutaneously or intramuscularly (Dhuner & Lewis 1966) A spe ulative reason for the decrease in intraocular pulse volume might be injection nduced mechanical compression in the orbit of limited volume The effect of drenaline on the results was unexpectedly small

The results obtained corroborate earlier observations of the lowering effect f RBA on IOP (Gifford 1949 de Roetth & Carroll 1955 Everett et al 1959 'ey et al 1962 Miklos & Halmai 1964 Syrdalen & Hørven 1970) The con radiction to a recent study in which no fall was established in IOP remains nclarified (Gjotteberg & Ingemansson 1977)

The reason for the decrease in IOP in connection with RBA is not known Aqueous outflow does not improve after RBA and it has been thought there ore that what is in question is reduced formation of aqueous due to the locking of the ciliary ganglion (de Roetth & Carroll 1955) Relaxation of the culomotor muscles has been proffered as one possibility (Kettesy 1967) At inson (1934) believed that the fall in IOP is caused by arterial constriction vhen epinephrine is used The same opinion was held by Gartner (1959) who onsidered that there may also be a reduction of aqueous secretion due to the onstriction of the vessels supplying the ciliary body

The results of the present work support the view that RBA lowers IOP at east partly by a direct vascular effect It is not necessary to add adrenaline o the anaesthetic to produce this effect The reduction in the blood supply robably causes a decrease in the quantity of intraocular blood The free anastomosis of the venae vorticosae and other orbital veins with the facial ein would allow free exit of the blood (Atkinson 1934)

Adrenaline added to the anaesthetic agent had such a small effect on the ntraocular blood supply that its use with RBA is not of great significance in his respect However when an eye with poor circulation needs RBA it is safer o use anaesthetic agent without adrenaline

References

Atkinson W S (1934) Local anesthesia in ophthalmology *Trans amer ophthal* : 32 399–451

Bynke H G (1968) Influence of intraocular pressure on the amplitude of the cor pulse *Acta ophthal (Kbh)* 46 1135–1145

Dhuner K G & Lewis D H (1966) Effect of local anaesthetics and vasoconstr upon regional blood flow *Acta anaesth scand Suppl* 23 347–35?

Everett W G Vey E K & Veenis C Y (1959) Factors in reducing ocular ten prior to intraocular surgery *Trans amer Acad Ophthal Otolaryng* 63 ?56-

Gartner S (1959) Methods of inducing anesthesia and hypotony for cataract surg *Arch Ophthal (Chicago)* 61 50–54

Gifford H (1949) A study of the effect of retrobulbar anesthesia on the ocular ten and the vitreous pressure *Amer J Ophthal* 32 1359–1368

Gjotterberg M & Ingemansson S O (1977) Effect on intraocular pressure of re bulbar injection of xylocaine with and without adrenaline *Acta ophthal (Kbh)* 709–716

Kettesy A (1967) Glaskorperverlust Haufigkeit Bedeutung Ursachen und Verhu *Klin Mbl Augenheilk* 150 785–804

Langham M E (1974) *The application of the Alcon applanation pneumatonograph clinical ophthalmology* Alcon Laboratories Forth Worth

Miklos A & Halmai O (1964) Verringert retrobulbare Injektion oder digitale K pression die Haufigkeit des Glaskorperverlustes bei Staroperationen? *Klin Mbl genheilk* 144 92–115

Moore D C (1955) Local toxic reactions to drugs used in regional block In *C plications of regional anesthesia* pp 93–111 Charles C Thomas Springfield

de Roetth A Jr & Carroll F D (1955) Effect of retrobulbar procaine injection aqueous humor dynamics *Arch Ophthal (Chicago)* 53 399–403

Stallard H B (1973) *Eye surgery* p 59? 5th ed John Wright & Sons ltd Brist

Syrdalen P & Horven I (1970) Corneal indentation pulse and retrobulbar anaesth *Acta ophthal (Kbh)* 18 719–730

Vey E K Finlay J & Everett W G (1962) A clinical evaluation of mepivacaine retrobulbar anesthesia *Amer J Ophthal* 53 827–83?

Author s address

Pekka Pohjanpelto
Honkapirtinkatu
15900 Lahti 90
Finland

Department of Ophthalmology (Head B Tengroth) and*
*Department of Clinical Alcohol and Drug Research (Head C M Idestrom)**
Karolinska Institutet Stockholm

THE EFFECT OF OXAZEPAM ON OCULAR READAPTATION TIME

BY

H BERGMAN * S BORG B HÖGMAN*
H LARSSON** C J LINDE and B TENGROTH*

The readaptation time (RAT) is the interval during which a person exposed to a bright intense light flash cannot perceive a given target In this study the target used was an optokinetic pattern and the elicited nystagmus (OKN) was registered with electrooculography (EOG) thus giving an objective registration of RAT Oxazepam in therapeutic doses was given to five healthy subjects and the RAT and serum concentrations of the drug were registered simultaneously at different time intervals An almost parallel increase of RAT and serum concentration of oxazepam was recorded This suggests that RAT reflects the depressant effect on the CNS of this drug and it may be used as an objective method of following the clinical effect of a depressant drug as a function of time after intake

Key words optokinetic nystagmus (OKN) – readaptation time (RAT) – oxazepam – photo stress

Among the problems in the evaluation of the hypnotic and sedative effects of drugs are the difficulties in finding methods which are sufficiently sensitive and of obtaining objective recordings of these

In this study registration of ocular readaptation time (RAT) following a

Received August 28 1978

brief exposure to glare is utilized The method was used in a previous r... to register effects of alcohol on healthy human subjects (Hogman et al 19

The aim of the present study is to determine whether effects on RAT ca.. k recorded after intake of oxazepam in clinical doses It also examines the fe sibility of using RAT to register the effect of oxazepam on the human cent nervous system (CNS) including the retina as a function of time after intak

Material

Five healthy male subjects aged 26–33 years volunteered for the study of them had consumed drugs during the previous four weeks before investi tion and smoking was not allowed during the experiment

Methods

Recording of readaptation time

Readaptation time (RAT) was determined by registering the pause in opto kinetic nystagmus (OKN) that appears after photo stress using electro-ocul graphy (EOG)

An optokinetic pattern is presented covering 25 % of the subject s field o vision The glaring light source covers a somewhat larger area The lumina ties of the background and of the glaring light are constant (10^{-4} cdm^{2} an 10^{4} cdm respectively) The method has been presented elsewhere (Tengrot et al 1976) First an initial recording was conducted on every subject at 8 a m to establish individual reference values for the subsequent registrations afte drug intake thus after intake of 20 mg oxazepam (Serepax®) per os RAT wa measured after 15 and 45 min Thereafter once every 30 min up to 195 min and then once every 60 min up to 405 min Venous blood samples were draw 30 45 75 195 315 and 435 min after intake

Statistical analysis

Each RAT value used in the statistical analysis was the median of five separa measurements made at intervals of 1 min RAT changes at different points of tim were tested for significance in a one way repeated measures analysis of variance de sign A trend analysis using orthogonal polynomials was also performed (Winer 19 In order to obtain an equal interval time scale the RAT value registered 30 min af r intake was excluded from the analysis A P value less than 0 05 was considered b logically significant in this study

termination of oxazepam in plasma

kalized plasma was extracted with benzene (containing 1 5 % v/v isoamylalcohol) ter centrifuging an aliquot (?–5 µl) of the clear supernatant was analyzed by gas uid chromatography using a Varian Aerograph 1400 equipped with a ^{63}Ni detector internal standard 2 amino 5 chlorobenzophenone (ACB) was used
ie chromatographic column was 6 ft glass packed with 1.5 % OV 17 on Chromo b WHP (80-100 mesh) Carrier gas was nitrogen, ultra pure gas flow 30 ml/min. mperature of the injector was 290 C of the column 2?5 C and of the detector 0 C Under these conditions the retention times were 2 3 min for ABC and 5 6 min oxazepam. The calculations of the oxazepam contents of the samples were per med by a standard graph in which the peak height ratios $\frac{\text{oxazepam}}{\text{ACB}}$ were plotted ainst known concentrations of oxazepam

x

Results

ie changes in RAT at different points of time after intake of 20 mg oxa pam (Serepax®) are shown in Fig 1 and the results of the statistical analysis Table I There was a statistically significant change of RAT mainly due the non linear components of the trend over time Maximal RAT prolonga on of the group was recorded after 2 h and the base line was reached ap oximately 5 h after intake A considerable inter individual variation of the aximal prolongation of RAT was observed – from ?5 min to 165 min after

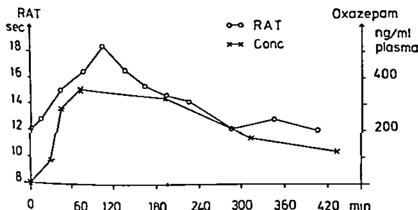


Fig 1

Readaptation time and plasma concentration of oxazepam after intake of 20 mg Serepax® Mean values of five healthy volunteers

Table 1

One way repeated measures polynomial analysis of variance of RAT at various t after intake of 20 mg oxazepam (Serepax®) per os

Source	df	MS	F
T (times)	7	24 23	3 6?
T × Subjects	28	6 70	
T (linear)	1	37 72	3 6?
Error	4	10 33	
T (quadratic)	1	50 04	14 83
Error	4	3 36	
T (cubic)	1	56 59	9.55*
Error	4	5 9?	

* $P < 0\ 05$ ** $P < 0\ 01$

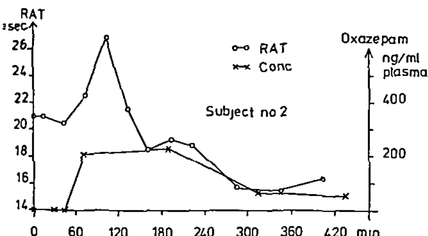
T = points of time after intake df = degrees of freedom MS = mean square F F r

intake Changes of drug concentrations in the blood also exhibited considera inter individual variation with maximal values noted from 45 min to 1½ after intake (Fig 2) The changes in RAT followed the changes of oxaze concentration in the blood during the ascending phase Thus in subject where the oxazepam concentration rose slowly the maximal change in R was recorded later than in subject 3 who had a faster rate of absorption

Discussion

The effect on RAT is in agreement with the effect on critical flicker fu (CFF) which also has been shown to be sensitive to 20 mg oxazepam pe given to healthy volunteers (Molander & Duvhok 1976) The maximal ef on CFF was shown after 3 h and the effect lasted for about 5 h In the pres study the maximal effect was recorded after 2 h and the effect lasted for a

In a previous study RAT prolongation was recorded after alcohol in and was shown to be prolonged during the acute intoxication phase as wel during the hangover phase (Hogman et al 1977) The observation that R was prolonged after intake of oxazepam in the present experiment supports assumption that RAT reflects effects on the CNS Furthermore the experi shows that it is possible to register the effects of CNS depressants in th



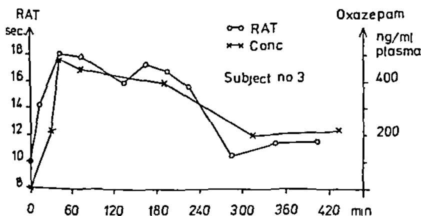


Fig °

Readaptation time and plasma concentration of oxazepam after intake of °0 mg Serepax® in two of the volunteers

-utic blood concentrations The present study also indicates that RAT might ;e used to follow clinical manifes.ations of CNS depressant drugs as a function ,i time after intake

Acknowledgment

ıe study was supported by grants from Svenska Lakaresallskapet and Karolinska ıtitutet.

References

Hogman B Bergman H Borg S Eriksson T Goldberg L Jones A W Linde C & Tengroth B (197/) Readaptation time after photo stress Alcohol induced and post alcohol hangover changes in ocular readaptation time *Psychopharmacology 53* 165–167

Molander L & Duvhok C (1976) Acute effects of oxazepam diazepam and m perone alone and in combination with alcohol on sedation coordination and *Acta pharmacol et toxicol 38* 145–160

Tengroth B Hogman B Linde C J & Bergman H (19/6) Readaptation time photo stress Readaptation time as a function of oxygen concentration *Acta opht (Kbh) 54* 50/–516

Winer B J (1971) *Statistical principles in experimental design* McGraw Hill York

Author s address

B Tengroth M D
Department of Ophthalmology
Karolinska sjukhuset
S 104 01 Stockholm
Sweden

Department of Experimental Ophthalmology (Head C E T Krakau)
University Eye Clinic Lund Sweden and
Dalby Community Care Research Centre (Head Ake Norden) Lund Sweden

A SIMPLE ROUTINE FOR OPTIC DISC PHOTOGRAPHY THROUGH A NATURAL PUPIL

Short Communication

BY

BO BENGTSSON and C E T KRAKAU

A simple device is described which made it possible to obtain usable fundus pictures in ca 95 per cent of 2000 eyes without dilatation of the pupil The camera is set to the patient s refraction by means of a scale on the camera extension

Key words fundus photography – Zeiss Fundus Camera – natural pupil

The examinations included in a population survey must neither be very time consuming nor disagreeable to the patients Participants must not be frightened from coming back, if necessary nor must they warn fellow patients not to join the survey

In a population study at Dalby on the secondary prevention of glaucoma it was found highly desirable to document the ophthalmoscopic findings by fundus photography In a previous survey however the discomfort caused by mydriatic drops resulted in numerous complaints

For this reason we found it worth while to try a procedure not totally lege artis namely to photograph without artificial dilatation of the pupil Our aim was merely to produce a picture sufficiently sharp to allow us to estimate the general shape of the papilla and its degree of excavation

Received September 15 1978

Method

1 Focusing

Since the camera extension is linearly related to the patient's principal p[…] refraction a focusing scale can easily be constructed and attached to the c[…]mera (Fig 1) (Bengtsson & Krakau 1977) A refraction value is obtained […] testing by Donders method and the camera is set at this value before expos[…]

2 Fixation

The subject is asked to fix a dim light situated about 15 degrees temporal[…] the second eye.

3 Adjustment of the camera position

The light from the flash tube of the Zeiss camera is conveyed into the examined […] by a mirror behind the front lens Rays from the fundus reach the photographic […] through a central hole in this mirror When the camera is in the proper position […] hole of the mirror is imaged by the front lens on the surface of the cornea. Conversely if the hole is illuminated from behind and its image brought to sharpness on the co[…] surface, we have obtained the correct distance between the camera and the examined eye.

The determination of this position is greatly facilitated by the following simple device. A tube containing a lens and two small electric bulbs is m[…] on the eyepiece of the camera (Fig 2) The bulbs are placed in the focal pl[…] of the lens and as far apart as possible in order still permit their beams to p[…] backwards through the camera. As a consequence, two widely separated pe[…]

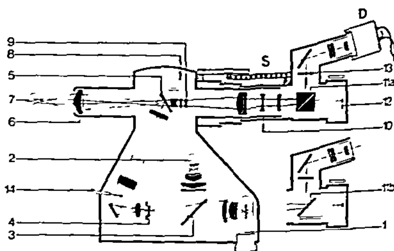


Fig 1
Diagram of Zeiss fundus camera, with scale (S) and adjustment device (D) a[…]

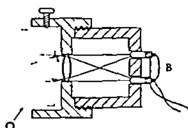


Fig 2
Adjustment device mounted on camera ocular (O) B Filament bulbs

f light emerge from the front lens intersect forming a single sharply demar ited spot of light on the corneal surface of the examined eye and enter the upil – again somewhat separated – provided that the camera is properly igned Even a slight error in adjusting the distance from the examined eye the camera is revealed by a doubling of the light spot on the corneal surface the camera is displaced in the frontal plane the iris becomes brightly illu inated

Exposure

he spot lights used for alignment of the camera are extinguished and after short delay the shutter is tripped Thus the pupil is allowed to dilate during dark period preceding the flash The retina surrounding the disc has to be ept under exposed in order to avoid over exposure of the disc The weakest lash (60 Ws) is sufficient

When the camera is used in this way the original filament bulb for fundus llumination has to be removed and the optic disc is photographed blindly in he sense that the eye ground is not observed at all during the whole operation

Results and Discussion

y this method the camera is carefully adjusted and the exposure made in ess than 30 sec This is true even in the presence of moderate ocular opaci ies which often cause a tiresome prolongation of the conventional procedure n elderly persons

Our aim is merely to obtain a documentation of the papillary structures uperior to drawings and verbal descriptions This modest requirement is ful illed in a surprisingly high percentage – no less than 95 per cent of all pic ures – about 2000 – allowing reliable measurements of cup and disc sizes even n elderly persons

In our opinion the simplicity and dependability of the method renders it well suited for recording of ophthalmoscopic findings at repeated eye examinations Since the pupil is not dilated but somewhat constricted by the light at adjustment the scope within which the imaging rays can be decentered is small The parallactic variation between different pictures from this eye is therefore limited Similarly the effects of imaging the disc peripherally are largely avoided when the subject fixes his gaze correctly

Reference

Bengtsson B & Krakau C E T (1977) Some essential optical features of the Zeiss fundus camera *Acta ophthal (Kbh) 55* 123–131

Authors addresses

Bo Bengtsson med lic
Vårdcentralen
S 240 10 Dalby
Sweden

C E T Krakau professor
Dpt of Exp Ophthalmology
University Eye Clinic
S 221 85 Lund
Sweden

Bech Knud & Jensen Ove Aksel External Ocular Tumours A Clinicopathologic Study of 300 Cases Textbook and Atlas Georg Thieme Publishers Stuttgart and W B Saunders Philadephia London, Toronto 1978 64 pages 116 figures Price DM 30 –

The book is a real ophthalmological gem for the ophthalmologist who is not especially experienced in the field of tumours It describes in an easily read and concise text with abundant illustrations the many different types of tumour of the outer eye The illustrations are of an exceptionally high standard and comprise more than half of the book Forty seven out of the 300 patients examined are reproduced in a most didactic fashion, whereby each individual case is demonstrated by means of a clinical a macrohistological and a microhistological photograph

The short text covers the main clinical and histopathological features in the course of 11 and three pages respectively 236 out of 300 patients had skin tumours while the remaining cases were equally divided between palpebral conjunctiva bulbar conjunctiva and caruncle

As the tentative diagnosis were correct in only two thirds of the cases the authors recommend that any tumour or tumour like lesion be examined histologically Clear and simple instructions are given as to when a marginal biopsy should be made and when primary total excision is preferable

The book can be highly recommended to any clinician requiring easy concise and clear information about the common tumours of the outer eye

E Gregersen

Handbook of Sensory Physiology Editorial Board H Autrum R Jung W R Loewenstein D M MacKay and H L Teuber Vol 7 part 5 The Visual System in Vertebrates Editor F Crescitelli Dept of Biology University of California Los Angeles Springer Verlag Berlin Heidelberg New York 1977 pp 813 Figs 284 Tables 93 Cloth DM 340 US $ 149 60 Subscription price Cloth DM 2?? US $ 119 70

This is the fifth part of volume seven of this comprehensive handbook Volume seven is dealing with all aspects of the physiology of the visual system In 11 chapters each written by an expert in his field the present part describes the vertebrate eye through the evolution starting with the cyclosome retina and ending with the topography of vision in mammals of contrasting life style Except for mammals the reader is not informed much about the visual pathways The vertebrate eye and its physiology had therefore been a more appropriate title

The first chapter on the history of vertebrates is very valuable for the non palaeontologist pointing out the many unsolved questions in the evolution of vertebrates Two chapters concern the fish eye and its adaptations to deep sea and photic environments and two chapters the amphibian eye Through the eyes of geckos and turtles and their visual world the reader is condu ted to the fascinating avian eye in so many respects more perfect than the human The book shows how justified it is to consider

The Jules François Prize

This prize will be awarded every three years to a research worker who has made an important contribution to ophthalmology The candidates must be not older than [illegible] years of age on the first January 1980 The amount awarded will be 100 000 BF

The candidature must be sent to the secretary of the Jules François Foundation [illegible] the candidate himself or a third person accompanied by all the necessary documents for justification Each candidature must be sent in before the first January 1980

Secretary J D Haenens M D E Beernaertstraat 34 8400 Oostende Belgium

3 Glaukom Symposium DDR

Wird in Neubrandenburg (zwishen Rostock und Schwerin) gehelt vom 9 bis [illegible] 1979 Hauptthema Das Sekundar Glaukom

Interessenten werden sich an Prof G Pietruschka DDR 25 Rostock Doberaner str 140

The European Ophthalmic Pathology Society

held its Annual Meeting in Belgrade Yugoslavia June 14–17 1978 Dr A P Fe[illegible] (Richmond Virginia USA) was the guest of honour The scientic program included 39 case presentations by members and guests and 17 nations were represented. For each presentation a protocol histopathological sections and appropriate clinical macroscopic transparencies were provided

The majority of case presentations dealt with benign or malignant neoplastic processes involving the globe orbit or eyelid and the discussion was concerned predominantly with classification prognosis and management Congenital malformations were also a prominent feature among the presentations and the remainder of the cases provided examples of systematic disorders involving ocular or adnexal tissues

Dr P Dhermy (France) was elected President and Dr W R Lee (UK) [illegible] elected Corresponding Secretary of the Society

International Society for Clinical Electrophysiology of Vision

The International Society for Clinical Electrophysiology of Vision will hold its [illegible] Symposium in Schloss Reinhardsbrunn Friedrichroda German Democratic Republic 5–10 June 1979

Two topics have been selected Visual electrodiagnosis in systemic diseases [illegible] Visual electrophysiology and localised retinal stimulation The official language of the Symposium is English

For further information and registration write to

Professor Dr E Schmoger Augenklinik der Medizinische Akademie Nordhäuser[illegible] 74 50 Erfurt GDR

From the Department of Ophthalmology (Head Salme Vannas)
University of Helsinki

A SIMPLE METHOD FOR SCREENING OF CHILDREN WITH STRABISMUS ANISOMETROPIA OR AMETROPIA BY SIMULTANEOUS PHOTOGRAPHY OF THE CORNEAL AND THE FUNDUS REFLEXES

BY

KARI KAAKINEN

A simple screening method for detecting strab smus anisometropia and ametropia in young children by simultaneous photography of the corneal and fundus reflexes with a conventional camera and flashlight is pre sented The method is the photographic application of the von Bruckner Durchleuchtung test and static skiascopy The objective document from the external part of the eyes and the face is obtained at the same time Model photographs with certain gaze deviation angles are presented with the method

Key words strabismus – von Bruckner Durchleuchtung test – static skia scopy – fundus reflex – corneal reflex – photography screening of vision defects and eye disorders in young children – flashlight – flickering fixa tion light (LED) – simple documentation

is important for the prevention of amblyopia to make a strabismus diagnosis early as possible in childhood (for example Pigassou (1977))
Ingram (1977) pondered the problem of screening children for visual de cts In his opinion the methods used today are still time consuming and xpensive Especially straight eyed amblyopes with anisometropic refractive rors require determination of refraction in early childhood Unfortunately xaminations at this age can often be very difficult even impossible because f fear or some other lack of co operation on the part of the child Even the over test can be very difficult to perform when the subject is under three

Received June 6 1978

years There is no simple and quick screening method to detect this kin visual defect

Von Bruckner (1962 1965) presented the Durchleuchtung test in which corneal reflexes according to the Hirschberg test and the fundus reflexes r pupillary reactions are also examined with an ophthalmoscope from a dist of one metre The strabismus or anisometropia can be diagnosed from asymmetry of these reflexes without touching the child It seemed poss therefore to make the documented von Bruckner Durchleuchtung test by a conventional camera with a flashlight instead of an ophthalmoscope that is required is for the child to look at the camera for the brief mom when the corneal and fundus reflexes are photographed The photographs c be later analysed by an ophthalmologist for corneal or fundus reflex asy metry indicative of strabismus or anisometropia In addition the fundus r flex photographs could provide information on possible changes in transp ocular media The approximate refractive errors too can be estimated f the different appearance of the fundus reflexes (Rosengren 1937 1945, a cording to the static skiascopy

Maddox (1902) used photography to examine strabismus angles Later an others Graham & Naylor (1957) Weekers et al (1963) Joachim & Gl (1964) Breitenmoser & Wurth (1970) and Jones & Eskridge (1970) ph graphed the Hirschberg test and determined the stage of the deviation of t eyes from the localisation of the corneal reflexes

Transparent ocular media are also examined quite frequently by pho graphy generally using the Douvas & Allen (1950) principle This has b applied in practice by Fincham (1955) and many others

Howland & Howland (1974) presented an apparatus for photorefractio both eyes with a camera but I have not found any report in the literature the use of the combined corneal and fundus reflex photography method both eyes for diagnosing strabismus and refractive errors simultaneously

To discover if this was possible I constructed a simple photograph equipment for simultaneous documentation of the corneal and fundus refle and to test its practicality in diagnosing strabismus and refractive errors

The first part of this investigation presents primarily the method and ex experience gained with its use

Methods and Material

In principle it is possible by using a coaxial flashlight attached to the o jective of the camera to photograph simultaneously the corneal and fu reflex when the patient fixes his gaze on the camera objective

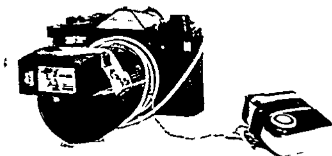


Fig 1

The pocket flashlight Sunpak Gx 17 in front of the Canon objective FD 100 mm 2 8 S S C The red flickering fixation light (LED) is in the middle under the flash unit On the right is the oscillation circuit box of the flicker system with a switch on coupler This was usually mounted in the back of the camera body

A standard 35 mm single lens reflex camera (Canon EF) with an FD 100 mm 1 2 8 S S C Canon lens was used A weak telephoto lens was chosen because it is quite small and handy but still gives enough enlargement from a distance of one metre to detect even slight deviations of the eyes

A Sunpak Gx 17 electronic flash unit was attached in front of the objective in the middle horizontally with the help of the Canon lens hood BT 55 so that the distance between the low edge of the flash unit and the lowest outside margin of the lens hood was 42 mm A piece corresponding to the size of the flash unit was cut from the lens hood so that the flash unit was firmly attached by the hood

A commercial red round flickering LED 9 5 mm in diameter was attached as a fixation light to the middle of the anterior low edge of the flash unit

The flickering mechanism was accomplished with an oscillation circuit The light was connected to a switch which was placed at the back of the camera body for easy handling (Fig 1)

The film material was commercial colour films The aperture range was adjusted to the Sunpak Gx 17 flash units guide number chart for a photography distance of one metre which was selected as the practical distance for photography This is the same distance that is generally used for the von Bruckner Durchleuchtung test

The photography technique and standardisation

To prevent inadvertent movement of the distance scale adjustment of the objective it was fixed with a piece of adhesive tape at exactly one metre Photography was done by hand without a stand For focusing the camera was slowly moved to and fro until the object in the middle of the viewer was sharpest In repeated experimental measurements focusing in this way at one metre with the camera s range finder the greatest possible error was ± 2 cm

The photography was performed in dim light for reliable focusin T pupils of the subjects were then larger than in bright light and the flicke fixation light interested the children and tended to make them stare at it

Mydriatic drops were not used because the aim was to photograph t physiological situation With young children in particular it was found use to focus first and then switch on the flicker light just before taking the ph graph asking at the same time what was flickering This gave both a v and auditory stimulus to the child to fix his gaze on the light

Te ting the method

1 *Summary measurement of refraction by photography* The usefulness of t method was examined by photographing a Carl Zeiss Jena Optical Demonstr tion Eye with different refraction errors which were controlled with a str retinoscope (Fig 2)

2 *Detecting strabismus* To establish the smallest deviation angle of the e that causes the change in intensity or colour of the fundus reflex or that c be seen as shifting of the corneal reflexes in the photographs an experimen test photo series was made An emmetropic straight eyed test subject w photographed when her gaze deviated from the fixation light of the camera a certain number of degrees (1°–13°) The deviation angles were calculated fr a slightly coarse cyclopean eye schema by using trigonometrical tangent fun tions in a right angled triangle where both the catheds were known t greater was the photography distance of one metre and the smaller the mea ured distance that the test subject was asked to look to the side of the fixat light of the camera

By calculating trigonometrically it is possible to determine by using t kind of cyclopean eye schema instead of the two eyed natural situation t the error in 3° gaze deviation is ± 2 min of arc and in the case of 13° ± 6 m of arc This is accurate enough and the cyclopean eye schema can be used be cause it is easy and practical for making measurements (Fig 3)



Fig 2
Refractive error models photographed from the Carl Zeiss Jena Demonstration E (−5 0 −4 0 −3 0 −2 0 ±0 +2 0 +3 0 +4 0 and +5 0)

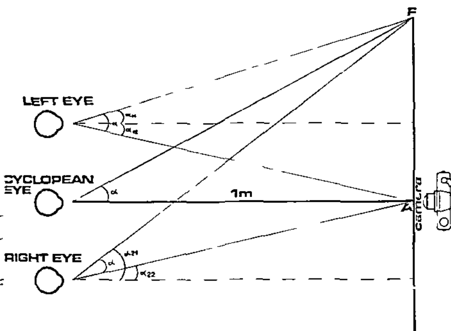


Fig 3

he gaze deviation angle of the cyclopean eye is denoted α and α_1 for the left eye nd α for the right eye in the corresponding natural situation

$$\alpha_1 = \alpha_{11} + \alpha_1$$
$$\alpha_2 = \alpha_{\ 1} - \alpha_2$$

$$[\alpha - \alpha_1] = [\alpha - (\alpha_{11} + \alpha_1)] \leq 0\ 6 \quad \text{when } 3 \leq \alpha \leq 13^\circ$$
$$[\alpha - \alpha\ ] = [\alpha - (\alpha_{\ 1} - \alpha_{22})] \leq 0\ 6 \quad \text{when } 3 \leq \alpha \leq 13^\circ$$

The photographs were taken twice and found identical When the gaze de iated one eye presented exotropia and the other esotropia The photographs ere compared with an orthophoric photograph where the same test subject azed into the camera (Fig 4)

To make the examination easier schemas corresponding to a normal strabis ius situation were made by cutting the photographs in the middle and com ining the gaze deviations of different degrees with half face photos in which ie test subject was looking straight ahead This gave artificial photos in which ne eye was straight and the other deviating a certain number of degrees Figs 5 and 6)

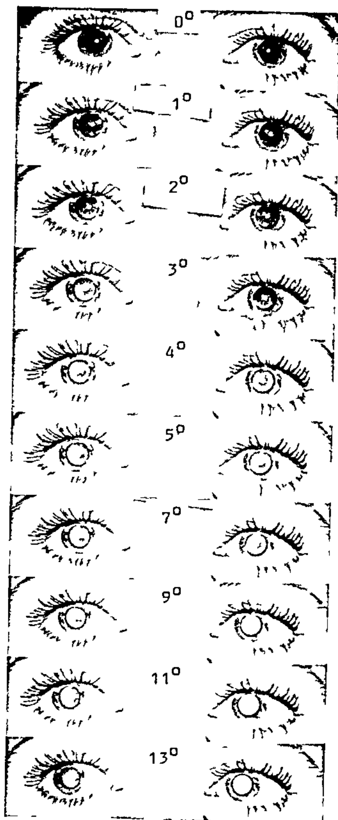


Fig 4 Photographed corn and fundus reflex when the gaze is deviated to the rig 1°–13° Top the gaz is fixed on the cam (0°)

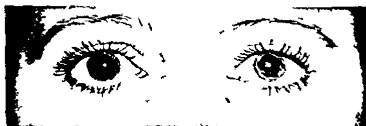


Fig 5

-tificial left esotropia 3° Combination of the two photographs The right eye is sed on the camera and the left 3 to the right of the camera The positions of the rneal reflexes are slightly asymmetrical The different brightness of the fundus reflexes is quite apparent

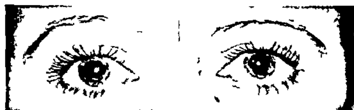


Fig 6

Artificial right exotropia 3° Combination of two photographs as in F g Moderate asymmetrical corneal and fundus reflexes

Results

he resolution and quality of the photographs was good In two c -ssions photographing the Carl Zeiss Demonstration I ye in d ion errors the results were identical

In hyperopia a light crescent was found in the low part of t nd in myopia in the upper part of the fundus reflex I ,t he myopic crescents increased in size with the refracti n hange could not be found when the small pupil of the ised The crescent appeared in myopia around -3 D a 1 diopters

In the test photos where the gaze of the test subj f degrees off the fixation light of the camera ngle of just 3 convergent or divergent strabismu ate deviation of corneal reflexes while at omewhat doubtful It was found that deviation

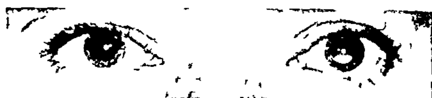


Fig 7
A 29 year old woman with anisometropia and left amblyopia Straight eyes in cover test Refraction in the cycloplegia in the right eye -3.5 comb cyl +0.5 ax 9 and in the left +5 0 comb cyl +1 0 ax 130° The vision of the right eye with correct. 1 6 and of the left 0 15 Anatomically normal eyes There are symmetrical corn. reflexes and the hyperopic crescent is visible in the left fundus reflex.

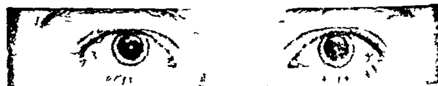


Fig 8
A 15 year old girl with -6 0 myopia in cycloplegia in both eyes Straight eyes in the cover test and anatomically normal eyes Symmetrical myopic crescents are visible in both fundus reflexes and the positions of the corneal reflexes are also symmetrical



Fig 9
A one year old boy with an anomaly of the right upper lid retraction and small intermittent hypotropia of the right eye Anatomically normal eyes and refraction of both eyes in cycloplegia The cover test reveals very small right hypotropia and sometimes no movement at all The right hypotropia and lid retraction are very apparent in the photograph The brightness of the fundus reflexes are symmetrical



Fig 10

10 year old boy with a very small left esotropia and amblyopia Refraction in the cloplegia on the right side +2 0 comb cyl +0 5 ax 90 and on the left +2 5 Ana nically normal eyes v o dx = 1 3 and v o sin = 0 1 with the correction In the ver test very small left esotropia in which the angle of the deviation with synopto ore is +9 A moderate asymmetry of the corneal reflexes is apparent in the photo aph and the fundus reflex of the deviating left eye is lighter than in the right one

ctable while 2 changes were demonstrable but somewhat questionable To termine the asymmetry of the corneal reflexes a normal millimetre scale as used at first to measure the distances of the corneal reflexes from the nbus But this was not practical because even the slightest asymmetry to be easured was at least as clear by visual examination

The colour of the fundus reflex became moderately lighter when the eye the test subject deviated from the straight position 1 medially or laterally hen the fundus reflex kept roughly as bright except at +11°–13 esodeviation hen it became very light coloured

In test photography sessions one year old children co operated by looking the fixation light Some illustrative strabismus and a straight eyed refrac ve error cases of different ages are presented in Figs 7–10

Discussion

a one year old child can be persuaded to co operate in dim light by looking t a flickering fixation light screening at that age could be possible with the ethod which because of its simplicity can even be set up by a technician

Graham & Naylor (1957) found that their eye deviation measurements made y photography differed from the synoptophoric results Joachim & Gilson 1964) reported that when they were examining by photography the measure ents by synoptophore gave overvaluation in convergent strabismus and under valuation in divergent strabismus These and the studies of Breitenmoser & Wurth (1970) and Jones & Eskridge (1970) give the impression that the devia ion angle of the eye at the moment of photography can be examined with a

great degree of accuracy from the photograph which at the same time is a permanent document for future reference

The different size of the kappa angles in the right and left eye of the same person could cause an error in estimating the possibility of strabismus by measuring eye deviations by comparing localisation of the corneal reflexes in the contralateral eyes But this source of error has been shown to be of minor importance in practice because photography has revealed that the kappa angles are identical in both eyes of the same person (Weekers et al 1963)

In principle the method described here is about as accurate for measuring eye deviations as the methods cited in the foregoing The procedure and apparatus are similar but simpler Complicated stands that could frighten a child are not needed The small and handy flickering LED light has shown itself to be a practical way of attracting the attention of small children

The method makes it possible to photograph with the conventional camera corneal and fundus reflexes simultaneously A mere 1° convergent or divergent deviation of the eyes in experiments made the fundus reflexes moderately lighter than in the straight position This concurs with the claim of von Brückner (1962) that a deviation of $1^1/_2°$ causes a change in pupillary brightness in the Durchleuchtung test It seems that this could facilitate the diagnosis of small-angled strabismus cases According to von Brückner (1962) the dark fundus reflex of the straight eye is reflected from the macula compared with the other parts of the retina which are lighter reflecting The very light fundus reflex that appeared in +11 –13° esodeviation is apparently caused by the bright reflex of the optic disc

It is clear that the method measures the refraction rather roughly according to the principle of static skiascopy in the line through the camera lens and the light source accordingly in the vertical meridian Its accuracy is not sufficient for exact refraction measurements and the method is meant mostly for screening not only to detect strabismus but also straight eyed cases with great anisometropic or ametropic refractive errors To obtain more accuracy for examining refractive errors especially hyperopia cycloplegic medication should be used

The method has already been applied to a clinical strabismus and refraction material that is to be published later

Acknowledgments

I am indebted to Docent Veikko Tommila for his critical support and to Paavo Hakkinen, M Sc for the mathematical planning and application

This study was supported in part by a grant from the Instrumentarium Research Foundation

References

eitenmoser R & Wurth A (19 0) Neue photographische Methode der Schielwinkel messung *Ophthalmologica 161* 346–35?

on Bruckner R (196?) Exakte Strabismusdiagnostik bei 1/--3 jahrigen Kindern mit einem einfachen Verfahren dem Durchleuchtungstest *Ophthalmologica 144* 184–198

on Bruckner R (1965) Praktische Uebungen mit dem Durchleuchtungstest zur Fruh diagnose des Strabismus *Ophthalmologica 149* 49 503

ouvas N & Allen L (1950) Anterior segment photography *Amer J Ophthal 33* 991–99?

encham E F (1955) Photographic recording of opacities of the ocular media *Brit J Ophthal 39* 85–89

raham P A & Naylor E J (1957) A photographic method of measuring the angle of squint *Brit J Ophthal 41* 4–5–433

owland H C & Howland B (1974) Photorefraction A technique for study of refrac tive state at a distance *J opt Soc Amer 64* 240–249

gram R M (19 a) The problem of screening children for visual defects *Brit J Ophthal 61* 4–7

gram R M (1977b) Refraction as a basis for screening children for squint and amblyopia *Brit J Ophthal 61* 8–15

achim M & Gilson M (1964) Étude comparative de la mesure des petits angles de strabisme au mouen de differentes méthodes *Arch Ophtal (Paris) 24* 533–538

nes R & Eskridge J B (1970) The Hirschberg test – a re evaluation *Amer J Optom and Arch Amer Acad Optom 47* 105–114

addox E E (190?) *Motilitatsstorungen des Auges* p ?16 Deidert s he Verlagsbuch handlung Leipzig

gassou R (1977) Entente cordiale in the early treatment of squint *Brit J Oph thal 61* 16–2?

osengren B (1937) A method of skiascopy with the electric ophthalmoscope *Acta ophthal (Kbh) 15* 501–506

osengren B (1948) A method of skiascopy with the electric ophthalmoscope *Acta ophthal (Kbh) 26* 215–?21

eekers R Gilson M & Joachim M (1963) Mesure des petits angles de strabisme au moyen d une technique photographique *Ophthalmologica 146* 217–2?6

uthor s address
an Kaakinen
alliokatu 15 B 15
?100 Kajaani 10
inland

The Department of Ophthalmology (Head Jan Ytteborg)
Ulleval sykehus Oslo

FUNDUS FLUORESCEIN ANGIOGRAPHY IN FUNDUS FLAVIMACULATUS AND STARGARDTS DISEASE

BY

NILS ANMARKRUD

Three siblings who had fundus flavimaculatus and two patients who had Stargardts disease were studied by means of fundus fluorescein angiography The angiograms revealed in all cases an abolished visibility of the chorioidal circulation New flecks are usually non fluorescent Later on hyperfluorescent areas are seen at identical places both in the pre retinal and retinal phases strongly indicating a window effect of the retinal layer The missing chorioidal flush is probably due to a blocking effect of the emitting and exciting light Some of the retinal flecks may fade away leaving corresponding areas of hyperfluorescence that usually persist In some cases however a previous fluorescent area may become non fluorescent The similar angiographic picture may indicate that fundus flavimaculatus and Stargardts disease are different expressions of the same disease

Key words fundus flavimaculatus – Stargardts disease – fundus fluorescein angiography – chorioidal circulation

Stargardt described in 1909 a bilateral hereditary macular degeneration curring in young people In addition to an atrophic macular lesion perimalar yellow white flecks were also noticed

Franceschetti (1963) introduced the term fundus flavimaculatus to sepa a special form of tapetoretinal degeneration In these cases the fundi w characterized by yellow white or yellow flecks at the level of the ret r l p ment epithelium in the posterior pole In 50 % of the patients Franceschi noticed an atrophic appearing macular degeneration Others have found a

Received October 16 1978

ilar degeneration in 67 % (Klien & Krill 1967) In these cases the distinction tween fundus flavimaculatus and Stargardts disease is difficult if at all ossible In the last years the view has been put forward that these two reti al degenerations are in fact different manifestations of the same disease This now commonly accepted (Deutman 1971 Irvine & Wergeland 1972 Fran ois et al 1975 Hadden & Gass 1976)

Fluorescein angiographic studies in fundus flavimaculatus were first men tioned by Ernest & Krill (1966) They concluded that the flecks and drusen were similar in location involving the retinal pigment epithelium

We have examined a family in which some of the members had reduced ision due to fundus flavimaculatus In addition we have examined two pa ents with Stargardts disease The purpose of this study is to describe the ngiographic pattern seen in these two diseases If Stargardts disease and undus flavimaculatus are different expressions of the same disease it would e of interest to study whether there exists a similarity in the angiographic icture

Material and Methods

The clinical material consists of 7 siblings (Fig 1) fair haired norwegian eople Their parents were healthy with no eye symptoms and no consanguinity Three of the siblings suffered from reduced vision due to fundus flavimacu atus In addition we have examined two patients with Stargardts disease

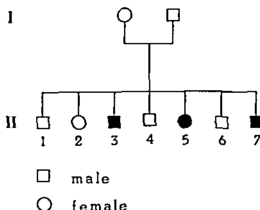


Fig 1
Pedigree of family with fundus flavimaculatus Black symbols indicate affected members

A complete ophthalmological examination was performed in every p... including fundus examination by miomicroscopy Goldman perimetry E... during scotopic conditions dark adaptation Ishihara colour screenin... fluorescein fundus angiography The angiograms were performed with a ?... fundus camera The film used was Kodak Plus X pan After injection of ... sodium fluescein solution (100 mg/ml) into an antecubital vein the ... grams were taken automatically every second during the transit of dye ... then 2 5 and 10 min after injection We used Zeiss interference filter KP... as excitation filter and Kodak Wratten No 15 as barrier filter

Case Reports

Case 1 (II 3) A 29 year old man developed bilateral decreased central vision at age of 22 years In 1973 visual acuity was 6/20 in right eye 6/1? in the left ... ERG and darkadaptation were normal He identified only the first of the Ishi... plates and a central scotoma was present in each eye Ophthalmoscopy reveale... normal optic disc and normal retinal vessels The macula in each eye showed a ... foveal atrophic lesion In the posterior pole many retinal yellow white fleck... various shapes and sizes were seen (Fig 2 A) Angiograms showed numerous hy... fluorescent areas both in preretinal and arteriovenous phases with abolished vis... of the choroidal circulation between these areas (Fig 2 B and Fig 2 C)

In 19/6 the visual acuity had deteriorated to c f 5 m right eye c f ? m left ... The atrophic macular lesions had increased in size but the yellow white fleck... of about the same number Some of the flecks had faded away while new ones ... been added (Fig 3 A) The angiogram showed increased number of hyperfluores... areas Some of the earlier yellow white flecks with no hyperfluorescence had fa... away and corresponding to these flecks the angiogram now revealed hyperfl... cence (Fig 3 B)

Case 2 (II 5) A 25 year old woman sister of case 1 developed decreased ce... vision in both eyes at the age of 14 years Visual acuity was c f 5 m in each ... ERG and dark adaptation were normal She identified only the first of the Ish... plates A central scotoma was found in each eye Ophthalmoscopy revealed a nor... optic disc and normal retinal vessels The macula in each eye showed an atro... pigmentary lesion with irregular margins Surrounding the macula were many yel... white flecks (Fig 4 A) Angiograms showed numerous hyperfluorescent areas ... abolished visibility of the choroidal circulation (Fig 4 B and Fig 4 C)

Case 3 (II 7) A 20 year old man the youngest brother of case 1 noticed re... central vision in both eyes at the age of 18 years Visual acuity was 6/1? in the ri... eye 6/20 in the left eye ERG and dark adaptation were normal He identified ... the first of the Ishihara plates and a central scotoma was present in each eye. In ... macula most pronounced in the left one were some deepseated yellow white f...

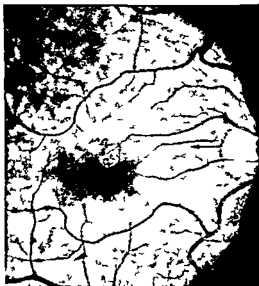


Fig 2 A

Case I 19 3 Right eye A An atrophic perifoveal lesion surrounded by numerous flecks B Angiogram (preretinal phase) showing numerous hyperfluorescent areas C Angiogram (arteriovenous phase) showing areas of hyperfluorescence of greater intensity than in B but the shape size and location are identical (Vertical arrows points to identical hyperfluorescent areas The long horizontal arrow demonstrates a retinal fleck showing fluorescence The short horizontal arrow demonstrates non fluorescent flecks) Note the abolished chorioidal circulation between the hyper fluorescent areas



Fig 2 B



Fig 2 C

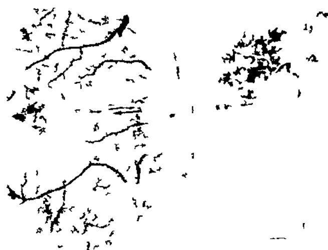


Fig 3 A Fig 3 B

Case 1 1976 Right eye *A* A marked atrophic lesion and numerous fleck are ... *B* Angiogram (arterio venous phase) showing more fluorescent areas than F 3 Since 19 3 a fleck has become non fluorescent (long horizontal arrow) and a pr... non fluorescent flecks now shows fluorescence (short horizontal arrow)

Most of them were round others with a more linear shape (Fig 5 A) The angio... (Fig 5 B) showed no hyperfluorescent areas and no visibility of the choroidal c... culation

In these three cases a diagnosis of fundus flavimaculatus was made The ... siblings were also examined All had normal retinal findings and normal f... angiography (Fig 6)

Case 4 A 24 year old woman in good health with no eye disorders in her ... noticed reduced central vision in both eyes at the age of 15 years Visual acuity ... 6/40 in each eye ERG and dark adaptation were normal She identified on... first of the Ishihara plates The macula in each eye showed an atrophic pigm... lesion with beaten margins No flecks were seen (Fig 7 A) The angiogram ... almost no choroidal flush and a hyperfluorescent macular area (Fig 7 B)

Case 5 A 46 year old man in good health with no eye disorders in his famil... ticed central vision of the age of 29 years Visual acuity was 4/40 in each ... central scotoma was found in each eye In each macula an atrophic lesion with ... margins was seen surrounded by some yellow white flecks Some of them were ... others were linear or pisiform with indistinct margins and a tendency towards ... fluence No flecks were seen outside the main vessels truncks (Fig 8 A) The ... gram showed abolished visibility of the chorioidal circulation hyperfluorescent ... area and hyperfluorescent areas in the macula (Fig 8 B)

In the last two cases a diagnosis of Stargardts disease was made

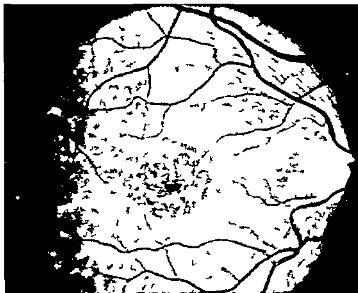


Fig 4 A

se 2 Right eye *A* An atrophic macular lesion surrounded by numerous flecks Angiogram (preretinal phase) showing numerous hyperfluorescent areas *C* Angio im arterio venous phase) showing areas of hyperfluorescence of greater intensity in in *B* but the shape size and location are identical (Vertical arrows points to ntical hyperfluorescent areas) Note the abolished choroidal circulation between the flecks



Fig 4 B

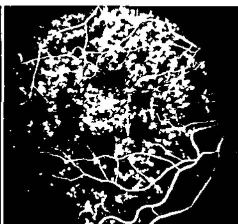


Fig 4 C



Fig 5 A Fig 5 B

Case 5 Left eye A In macula some deeply seated retinal flecks are present. B A-gram (arteriovenous phase) showing complete abolished visibility of the chor circulation but normal filling of the retinal vessels

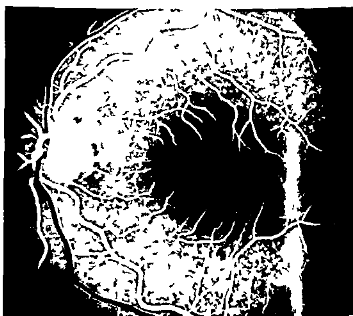
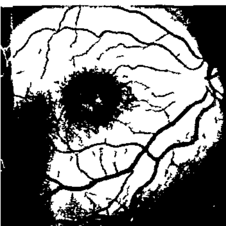


Fig 6

A healthy brother (II 4) in the family with fundus flavimaculatus Left eye A gram (early arteriovenous phase) showing normal angiographic pattern



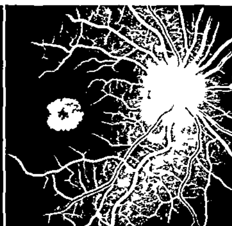
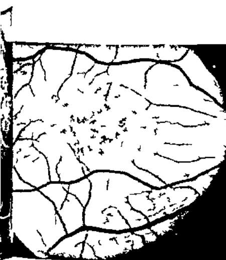


Fig A Fig B

Case 4 Right eye *A* An atrophic macular lesion with between margins no flecks Angiogram (arteriovenous phase) showing hyperfluorescence of the macular lesion and normal filling of the retinal vessels There is complete abolished visibility of the choroidal circulation



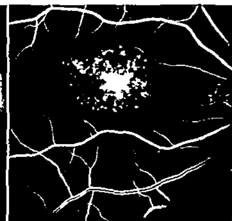


Fig 8 A Fig 8 B

Case 5 Right eye *A* A macular atrophic lesion with beaten margins surrounded by some flecks *B* Angiogram (arteriovenous phase) showing fluorescence of the atrophic lesion and and some of the flecks There is normal filling of the retinal vessels but complete abolished visibility of the choroidal circulation

Results

Changes in the chorioidal circulation

The three members of the family with fundus flavimaculatus demons- peculiar abnormal angiographic pattern abolished visibility of the chor- circulation This is most pronounced in case 3 (Fig 5 B) which is a ver- stage of the disease We also observed the same change in the chorioidal c- culation in case 1 (Fig 2 C) and case 2 (Fig 4 B) although the marked hy- fluorescence tends to mask it But between the hyperfluorescent areas the- no visibility of the chorioidal circulation Case 4 and case 5 which fulfil- criteria of Stargardts disease also show the same invisibility of the chor- circulation (Fig 7 B and Fig 8 B) The siblings not affected by fundus fl- maculatus had a completely normal chorioidal pattern although all were fa- haired with the same degree of retinal pigmentation (Fig 6)

Hypo hyperfluorescence of the retinal flecks

In the very early stage of fundus flavimaculatus only some minor yel- white flecks are seen in the macular area (Fig 5 A) The angiograms dem- strate very few changes except the missing chorioidal flush (Fig 5 B) As t- disease progresses more flecks are seen and the angiograms now reveal n- merous hyperfluorescent areas The flecks are usually found to be non fl- escent and the fluorescent areas in the angiograms are either seen between t- flecks or adjacent to the retinal flecks

The retinal flecks are often found to fade away (Fig 2 A and Fig 3 A- Corresponding to the previous fleck a hyperfluorescent area is now form- Since the retinal flecks may fade away and the hyperfluorescent areas us- persists a disproportion is made between the number of retinal flecks a- hyperfluorescent areas In some few cases we have seen a hyperfluoresc- area to disappear but this is unusual (Fig 2 C and Fig 3 B)

The areas of hyperfluorescence are seen before filling of the retinal arter- both in fundus flavimaculatus and Stargardts disease They have ide- shape size and location to those seen in the arterial and arteriovenous pha- of the angiograms although the intensity increases (Fig 2 B and Fig 4 C Fig 4 B and Fig 4 C) In no cases was late staining seen

Discussion

The abolished visibility of the chorioidal circulation is a peculiar in our pa- tients with fundus flavimaculatus and Stargardts disease The siblings no- affected by fundus flavimaculatus all had a normal angiographic pattern k-

ndus flavimaculatus and in Stargardts disease some alteration must exist th regard to the choroidal circulation The angiographic pattern could either due to occlusion of the choroidal circulation or something in front of the oriocapillaris that could obstruct the view of an otherwise normal choroidal rculation

In the preretinal phases of the angiograms numerous hyperfluorescent areas ere seen The location size and configuration were identical to those seen the retinal phases of the angiograms The fluorescent areas increased in tensity during the arterial and arteriovenous phases and faded away simul neously to normal choroidal fluorescence Between the hyperfluorescent eas no sign of choroidal fluorescence was seen The angiographic pattern the hyperfluorescent areas in the preretinal and retinal phases are highly iggestive of normal choroidal circulation which is seen through window fects in the retinal layers The invisibility of the choroidal circulation is robably due to a masking effect between choroidea and the retinal vessels he choroidal circulation is obviously present but is only partly seen through ie window defects

As far as I know only Bonnin et al (1976) have stressed similar alterations i certain posterior retinal degenerations and have called it le signe du lence choroidien

Klien & Krill (1976) made the only histopathologic examination of an eye ith fundus flavimaculatus They found distinctive changes in the pigment pithelium while neuroepithelium Bruchs membrane and choroid were nor ial The lesion appeared to be produced by an acid mucopolysaccharide lo ated inside the pigment epithelium The morphologic changes with displace ient of the nucleus toward the center of the cell and the peculiar line of con ensation of pigment granules near the inner surface of the cell can possibly xplain increased absorption of the emitting and exciting light A diffuse de osit of acid mucopolysaccharide will further increase absorption of the light

The abolished visibility of the choroidal circulation may thus be due to hysical problems concerning the filtering of the emitting and exciting light t is highly suggestive that a diffuse deposit at the level of pigment epithe ium Bruchs membrane or the outer s gment of the retina blocks the trans nission of light

The missing choroidal flush is not a constant sign in these two retinal liseases (Bonnin et al 1976) The reason for this is uncertain but the sign nay be related to various amount of diffuse deposits in the retina

New flecks are usually non fluorescent As the disease progresses they be come fluorescent The location size and shape of the hyperfluorescent areas strongly suggest a pigment epithelial window defect

Some of the flecks has been shown to fade away We have also found earlier hyperfluorescent areas can be non fluorescent although most of t persist The reason for this is uncertain

The similar angiographic picture with abolished visibility of the cho- circulation and time related angiographic changes of the retinal flecks be a further evidence that fundus flavimaculatus and Stargardts dise the same disease with a different expression

References

Bonnin P Passot M & Triolaire Cotton M (1976) Le signe du silence choroidien les degenerscences tapeto retiniennes posterieures In Le Lacy J J Ed Inter nal Symposium on Fluorescein Angiography pp 461–463 Dr J Wunk bv Pu lishers The Hague

Deutman A F (1971) *The hereditary dystrophies of the posterior pole of the ey* Charles C Thomas Springfield Illinois

Ernest J T & Krill A E (1966) Fluorescein studies in fundus flavimaculatus drused *Amer J Ophthal 62* 1–6

Franceschetti A (1963) Ueber tapeto retinale Degeneration im Kindesalter (kon tale Form (Leber) amaurotische Idiotie rezessive geschlechtsgebundene tap retinale Degenerationen Fundus albipunctatus cum hemeralopia Fundus fla culatus In Enke F Ed *Entwicklung und Fortschritt in der Au enheilkun* 10 –120 Enke Verlag Stuttgart

François P Turut P Puech B & Hache J C (1975) Maladie de Stargardt et f flavimaculatus *Arch Ophthal (Paris) 35* 817–846

Hadden O B & Gass J D M (1976) Fundus flavimaculatus and Stargardts d *Amer J Ophthal 82* 527–539

Irvine A R & Wergeland F L Jr (1972) Stargardts hereditary progressive ma degeneration *Brit J Ophthal 56* 817–826

Klien B A & Krill A E (1967) Fundus flavimaculatus Clinical functi n histopathologic observations *Amer J Ophthal 64* 3–23

Stargardt K (1909) Ueber familiare progressive Degeneration in der Makula ge des Auges *Albrecht v Graefes Arch Ophthal 71* 534–550

Author s address

Nils Anmarkrud M D
Eye Department
Ullevål sykehus
Oslo 1
Norway

Research Laboratory of Electronics[1]
Chalmers University of Technology (Head S Olving)
and Departments of Histology) (Head H Hydén)
and Ophthalmology[3] (Head E Linner)
University of Goteborg Goteborg Sweden

RETINAL DAMAGE EXPERIMENTALLY INDUCED BY MICROWAVE RADIATION AT 55 mW/cm

BY

L E PAULSSON[1] Y HAMNERIUS[1] H A HANSSON)
and J SJÖSTRAND[3]

The action of 5>0 W/m² (mean) 3100 MHz pulsed radiation on the rabbit retina *in vivo* was investigated by fundus photography blood retinal barrier tracers light and electron microscopy either after a single 1–1 5 h exposure or after a series of repeated 1 h exposures for up to 53 h during about 100 days

The electron microscopic investigation of the repeatedly exposed retinas revealed degenerative changes in the retinal neurons The neurons appeared depleted of their cytoplasmic constituents and often contained phago lysosomal structures with myelin bodies There wer many degenerating synaptic boutons The glial cells displayed reactive changes These ultrastructural changes could not be demonstrated by the other methods used A single microwave exposure followed by an induced /0–100 % blood pressure increase did not enhance blood retina barrier permeability to tracers There was no evidence of blood brain barrier leakage These studies show that the rabbit eye can be affected by microwave radiation at intensities lower than previously reported

Key words retinal damage – degeneration of synapses – microwave radiation – brain – blood retina barrier – blood brain barrier – ultra structure – fundus

The eye is one of the most well studied biological systems regarding the action of microwave radiation In clinical reports lens cataracts have been described as the most frequent hazard of microwave exposure in man Most experimental

Received September 9 1978

work on the effect of microwave radiation to the eye have been focused on induction of lens cataracts The action of microwaves on the posterior of the eye has received less attention Dailey et al (1948) reported re changes in dogs after high intensity exposure

In a clinical report in 1952 Hirsch (1970) described a man developing cataracts chorioretinal lesions and a scotoma following short term exposure to high intensity microwave exposure Recently Aurell & Tengroth (1973) reported high incidence of retinal changes among the personnel of a radar industry

Since our knowledge concerning the effect of microwave radiation on retina is very limited we have studied in the present work the action of 3 MHz pulsed microwaves on the rabbit retina A number of experimental methods were used to investigate the retinal morphology *in vivo* the retinal histology and the blood brain and blood retina barriers (BBB and BRB respectively) on animals subjected to either a single 1–1 5 h microwave exposure or to series of one h exposures repeated up to 53 times during a three month period

Materials and Methods

Animals

40 pigmented rabbits of the black and white Dutch breed of both sexes weighing 1 kg were used The microwave irradiations and the *in vivo* examinations were all performed without the use of any anaesthetic drugs

Microwave radiation

The microwave radiation was generated by an S band radar transmitter using a p magnetron capable of delivering a peak power of 250 kW at the frequency 3100 M The magnetron was coupled to an (IEC) R 32 waveguide which was terminated a horn antenna with aperture dimensions 157 × 135 mm in the E and H plane respectively (E plane horizontal)

A movable short was used to match the magnetron to the waveguide for obta high peak power and a reasonably good pulse shape The pulse length was fixe 1 4 μs and the pulse repetition frequency was 300 Hz The mean power outpu limited to 100 W by the power supply All irradiations were performed at a dis of 0 55 m between the rabbit head and the antenna aperture This is more than Fresnel distances to secure for far field conditions Walls of microwave absorbing terial was surrounding the rabbit at a distance of 1 m The rabbits were irra from the right side with the head in the center of the beam They were kept in a made of a polystyrene lattice and cooled by an air stream (2 5 m/s measured at position of the head) from a fan situated well outside the microwave beam

Dosimetry and temperature measurements

The mean output power of the transmitter was measured to 100 W The propert the antenna was determined separately and the microwave intensity at the d

ɔ m was calculated to ɔ/0 W/m The intensity could not be measured directly with power density meter because of the high pulse power Calorimetric measurements :h an ethanol filled spherical absorber calibrated against a power density meter gave a result an intensity of ɔ00 W/m A safe estimate of the mean intensity used would ɔɔ0 W/m ± 20 % with a pulse peak intensity of 1.3 10^6 W/m

The temperature increase in the rabbit eye orbit during the irradiation was deter ned in a separate experiment where the rabbit was given a light barbiturate an ithesia and a few drops of oxibuprocaine (0 4 %) as local anaesthetic. The rabbit was adiated for five min and thereafter the radiation was stopped A miniature thermo uple was immediately inserted below the eye bulb in the eye orbit beneath the center the eye and the temperature was registered The irradiation was then restarted This ocess was repeated for 60 min. The mean of measurements on two rabbits is given Fig 1

ndus photography (A)

ie rabbits irradiated repeatedly were examined once a week by fundus photography able I) The central part of the retina was photographed with a 3ɔ mm single lens flex camera adopted for macrophotography with a long extension tube and an in

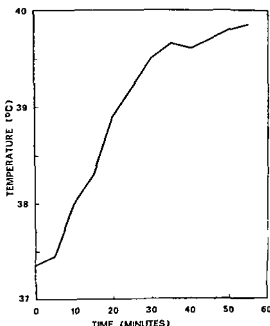


Fig 1

Temperature increase in rabbit eye orbit below the eye bulb during microwave irradiation (3 1 GHz 5ɔ0 W/m) The absorbed mean power density in the retina was about 3 10^4 W/m³ Mean value of measurements on two rabb ts

Table I
Experimental conditions

Experimental method	Exposure conditions			
	I	II	III	IV
A *In vivo* retinal morphology fundus photography	X			
B Light microscopy	X			
C Electron microscopy	X			X
D BBB inspection of brain	X	X	X	
E BRB fluorescence microscopy			X	

I Repeated exposures 1 h/day 3 d/week up to about 50 h
II Acute 1 5 h exposure
III Acute 1 h exposure followed by a drug induced 70–100 % blood pressure incr
IV Acute 1 h exposure killed after 3½ months

verted standard lens A small electronic flash illuminated the eye via a mirror outside the lens aperture The refraction of the cornea air surface was eliminated contact lens with a plane front surface (Goldmann lens Haag Streit AG Berne zerland) The animals were given a few drops of *Cyclogyl*® (1 %) to dilatate the p and oxibuprocaine (0 4 %) as a local anaesthetic A small amount of methyl ce secured good optical contact between lens and cornea The magnification of the ca was fixed at 5 × The resolution enabled observation of single bundles of mye nerve fibres Photographs were taken on 50 ASA colour slide film and viewed in parallel slide projectors by the investigators and others

Histology (B C)

Animals in groups I and IV (Table I) were killed and fixed by perfusion under p barbital anaesthesia As a fixative we used glutaraldehyde and formaldehyde in c dylate buffer as described by Karnowsky (1965) After perfusion 1 ml of the fix was slowly injected into the vitreous body of each eye An equal amount of fl was allowed to drain from a small incision at the corneal margin The eyes further fixed by immersion Parts of the central retina were postfixed in b osmium tetroxide dehydrated and embedded in Epon® For light microscopy sections were cut and stained with methylene blue For electron microscopy sections were cut with a diamond knife further stained with uranyl acetate and citrate and observed in an electron microscope

ood tissue barrier studies (D E)

ans blue and sodium fluorescein were used as tracers in blood barriers permeability ies Evans blue is strongly bound to serum albumin and the leakage of this dye ough the blood retina or blood brain barriers indicates barriers permeability to olecules of high molecular weight Sodium fluorescein is more loosely bound and dye penetrates more freely due to its lower molecular weight

In most experiments Evans blue (20 mg/kg) was injected as a 2 % solution in saline tered through a 0 47 μm Millipore® filter The injection was given 30 min following start of the 1 5 h exposure The animals were killed and fixed by perfusion under ntobarbital anaesthesia 70 min after the injection of the dye 4 % formaldehyde was ed as a fixative In those cases where sodium fluorescein was used the dye was in cted (25 mg kg) as a 10 % solution immediately after the irradiation and the animal is killed by an overdose of pentobarbital after 15 min without perfusion

The brain was dissected and the brain surface was inspected for the presence of dye akage In the case of sodium fluorescein the inspection was made under ultraviolet umination

All animals in the acute exposure group III (Table I) were subjected to an acute pertension induced by an injection of metaraminol as it was possible that some ani als might have experienced extreme stress accompanied with a hypertension during radiation and handling Such acute hypertension was shown by Blomstrand et al 975) to cause blood brain barrier leakage in X ray irradiated rabbits

The blood pressure was measured electromanometrically through a catheter inserted to the femoral artery The catheter was inserted under light pentobarbital anaesthe a During the surgery xylocaine® (1 %) was given as a local anaesthetic The ani als were allowed to recover from the anaesthesia and were able to sit normally during e irradiation The dye and the metaraminol (0 15 mg/kg) was injected immediately ter the irradiation

Fifteen min after the blood pressure increase the animals were sacrificed and the ain surface inspected as described above The right eye was enucleated and imme ately frozen in liquid propane transferred to liquid nitrogen and freeze dried va uum embedded in paraffin and sectioned for microscopy as described by Grayson & aties (19 1) The sections were observed in a fluorescence microscope

Results

he results are summarized in Table II

General observations

he animals were checked regularly and showed no changes in weight ap earance or motility as compared to the controls The pupillary reflex was hecked and the lens examined with a standard slit lamp No difference be ween control and irradiated animals was seen The appearance of the small vacuoles naturally located near the posterior sutures of the rabbit lens did not change during the repeated exposure experiment

Table II

Frequency of positive findings in rabbit retina and brain with different experimental methods (A–E) after acute (II–IV) or repeated (I) 550 W/m 3 1 GHz microwave exposures J means irradiated groups and C non irradiated controls

Method	Exposure I J	Exposure I C	Exposure II J	Exposure II C	Exposure III J	Exposure III C	Exposure IV J
A *In vivo* retinal morphology fundus photography	0[1)]/4	0/4					
B Retinal histology light microscopy	0/3	0/2					
C Retinal histology electron microscopy	3/3	0/2					[illegible]
D BBB inspection of brain	1/3	1/2	4/9	1/7	0/3	0/[illegible]	
E BRB fluorescence microscopy					0/5	0/3	

I–IV For explanation see Table I

1) The figure 0/4 means that in a group of 4 animals 0 showed any changes

Table III

Thickness of some retinal layers in two different positions in microwave irradiated and control rabbits The values are given in μm ± (standard error the mean ...

		Irradiated animals right	Irradiated animals left	Controls
Photoreceptor outer segments	pos 1	39 ± 4	40 ± 4	[illegible]
	pos 2	24 ± 1	24 ± 1	[illegible]
Photoreceptor inner segments	pos 1	18 ± 1	18 ± [illegible]	[illegible]
	pos 2	17 ± [illegible]	22 ± 3	[illegible]
Outer nuclear layer	pos 1	56 ± 1	55 ± 1	[illegible]
	pos 2	45 ± 3	47 ± 1	[illegible]
Inner retina extending from the outer plexiform layer to the internal limiting membrane	pos 1	9[illegible] ± [illegible]	10[illegible] ± 3	[illegible]
	pos 2	83 ±	89 ± 4	[illegible]

ndus photography (AI)

e fundus photographs from the repeatedly exposed animals were examined pairs with the aid of two slide projectors No signs of progressive changes in retina were observed

microscopy (BI)

ght microscopy of three different parts of the retina did not reveal any ob -vable differences between the irradiated animals and the controls The ickness of some clearly definable retinal layers was measured (Table III) l differences between the right and left eyes in the irradiated group or be een the mean values of all eyes in the two groups are all well within 95% nfidence intervals obtained from the t distribution This result indicated for ample that the processes of renewal and phagocytosis of the photoreceptor ter segment were not significantly affected by the radiation

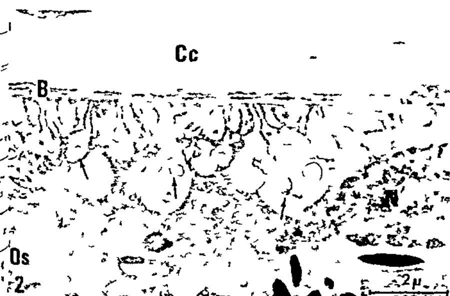


Fig 2

lectron micrograph of pigment epithelial cells (N) from a rabbit repeatedly exposed uring 3 months to microwave irradiation There is an increased accumulation of nembraneous material (arrows) in the dilated spaces between basal processes of the pithelial cells and the basement membrane (B) facing the choriocapillary layer (Cc) Outer segments (Os) of photoreceptor cells in the lower half of the figure. 10 000 x.



Fig 3

Electron micrograph of the outer plexiform layer of rabbit retina repeatedly
irradiated during 3 months The enlarged synaptic terminals are distorted
with numerous vesicles (Sv) Synaptic ribbons are marked by arrow heads. The
of the Mullerian (M) neuroglial cells form thin multiple lamella (arrows)
gliotic reactions db = dense body Rod = rod cell 16 000 x

Fig 4 a and b

4 a Electron micrograph of the same retina as in Fig 3 Dense bodies (db)
myelin figures are seen in synaptic terminals with only a few microtubules
ments retained There is an accumulation of fuzzy material (★) in several
A synaptic junction is marked by *arrows 2400 x*

4 b Electron micrograph of rabbit retina repeatedly microwave irradiated
3 months The synaptic terminals in the inner plexiform layers contain a
number of synaptic vesicles (arrow heads) There are two dense bodies
myelin figures (db in the synaptic terminals to the left A synaptic
seen in the upper right The Mullerian cell (M) shows reactive increase of
and glycogen granula 1

M

db

M

D

db

4a

5μ

Sy

M

db

4b

1μ

Electron microscopy (CI)

The electron microscopic investigation of the central retina in the right e[illegible] three repeatedly irradiated rabbits revealed a spectrum of changes The[illegible] an increased spacing between the basal processes of the pigmented ep[illegible] cells as well as a slightly increased accumulation of debris (Fig 2) The ph[illegible] receptors appeared normal with regard to the structure of their outer and [illegible] segments The synaptic terminals in the outer plexiform layer were [illegible] and markedly increased in size (Fig 3) Many of them contained very [illegible] synaptic ribbons and numerous dense bodies (Fig 3) The enlarged sy[illegible] terminals were filled with synaptic vesicles and contained only few other [illegible] ganelles Multiple layers of glial membranes were often observed bo[illegible] these synaptic terminals However in contrast the synapses in the inner p[illegible] form layer contained a reduced number of synaptic vesicles (Fig 4) De[illegible] bodies sometimes including myelin figures were frequently observed b[illegible] synaptic terminals and neuronal processes (Fig 4) The number of microt[illegible] and to a smaller extent filaments were reduced Several axons and den[illegible] contained fuzzy material (Fig 4)

Many of the ganglion cells showed an increased number of dense bod[illegible] well as of large vacuoles and of vesicles filled with fuzzy material (Fi[illegible] There was a loss of ribosomes profiles of endoplasmic reticulum and mitoc[illegible] dria too Spindle shaped dilatations of axons originating from ganglion c[illegible] were frequently observed as were similar axonal dilatations (Fig 5a) [illegible] minent gliotic reactions of mainly Mullerian neuroglial cells were seen in [illegible] layers of the retina especially at the posterior pole of the eye (Figs 3–5) The[illegible] was an increased number of filaments ribosomes and glycogen granules in the glial processes which sometimes formed multiple sheaths around the ne[illegible] processes (Figs 3 and 4)

Thus there were extensive changes in the majority of the neuronal proce[illegible] in the plexiform layers although there was a wide range in the degree [illegible]

Fig 5 a and b

5a Electron micrograph of the ganglion cell layer of rabbit retina in Fig 3 [illegible] ganglion cell (Gc) contains a vesicle filled with fuzzy material (+) The [illegible] glial cells (M) are reactive and filled with glycogen granules and filaments Some a[illegible] to the left (arrows) are dilated and filled with fuzzy material (★) 10 000 ×

5b Higher magnification of the nerve fibre layer adjacent to the vitreous body of [illegible] retina in Fig 3 Enlarged axons (arrows) filled with dense bodies (db) and [illegible] mentous material (f) are seen The axon (★) above is also enlarged but does not [illegible] play any true change in organelle distribution Mullerian cell process (M) for[illegible] complex pattern close to the basement membrane (bm) 22 000 ×


M
Gc
5a
db
M
f
M
5b
vitreous
bm

damage The most extensive changes were observed at the posterior pole J ever it should be stressed that many neuronal processes and cell bodies L significant changes

The left eyes of the animals irradiated from the right side and the having received less radiation were also examined The retina of these showed changes as described above However these changes were not exc but had to be considered significant in a subjective evaluation

Two rabbits (C IV) exposed once for 1 h and killed 3 5 months later ma changes in their right retinae similar to those described above for the le of rabbits subjected to multiple exposures

Blood brain barrier studies (DI DII)

The inspection for dye on the brain surface revealed some small (< 1 m coloured spots on some of the brains In the repeated exposure group (I animal showed two such spots and in the control group one animal showe spot In the acute exposure group (II) such spots were seen in 4 of 9 irra animals and in one of 7 controls

From statistical point of view no significant difference exists between irradiated and control groups at a 95 % confidence level

Blood brain barrier studies on rabbits with acute hypertension (DIII)

All animals in group III were subjected to a 70–100 % blood pressure i immediately following the irradiation and dye injection No signs of leakage was seen in either control or irradiated animals

Fluorescence microscopy (EIII)

About 7–10 sections from each right eye in the hypertension group were mined in the fluorescence microscope The red fluorescence of the Evans or alternatively the yellow green fluorescence of the sodium fluorescein seen with extreme intensity in the choroid and with decreasing intens wards in the sclera No traces of the fluorescent dye could be seen in tinal layers internal to the pigmented epithelium The sensitivity for de a weak fluorescence in the outer retina was somewhat lowered by the autofluorescence of the photoreceptors It was possible to discriminate betw the colour of this autofluorescence and that from the dyes by using viewing filters in the microscope

Discussion

threshold level of 1–1 5 kW/m² incident 2450 MHz microwave power for the rmation of lens cataracts in the rabbit eye is now generally accepted The ctron microscopic examinations in the present work reveal significant retinal anges after repeated 0 55 kW/m² pulsed 3100 MHz exposure The absence lens opacities showed that the power level used is not substantially higher an the stated 0 55 kW/m² These findings indicate that lesions observable at e electron microscopical level can be produced in the rabbit eye at power vels lower than the cataractogenic threshold No retinal change were seen owever by light microscopy and no leakage of fluorescent tracer dyes through e *blood retinal barrier was found This agrees well with the fact that no* anges could be observed with ophthalmoscopy and fundus photography The port of Hirsch (1970) on ophthalmoscopically observable retinal changes in man intermittently exposed to 1–9 kW/m² during three working days shows at such changes may be caused by high intensity microwave radiation The tinal changes on the personnel of a radar industry reported by Aurell & engroth (1973) were just detectable by a trained ophthalmologist equipped ith good instrumentation (Aurell & Tengroth personal communication) The posure history of these personnel was not very wellknown Many of them had en *working with microwaves for several years and it is likely that they were* xposed to intensities well below 0 1 kW/m for considerable times and that ort exposures to much higher intensities occurred occasionally It is reason ble to assume that the rabbits in the present work received microwave doses hich fall between the two cases described above both in time and intensity

The frequency dependence of the absorbed microwave power in the human ye was investigated by Paulsson (1976) It is seen from that study that the uman retina receives maximum power density at frequencies around 3000 MHz For the rabbit eye Guy et al (1975) have found retinal power densities –4 times *higher than that seen in the human eye* whereas *measurements on* rabbit irradiated under our conditions gave about 2 times Thus microwaves f frequencies around 3000 MHz are shown to be readily absorbed in the re ina in both rabbit and human eyes In the present work the absorbed mean ower density in the retina was about 3 10⁴ W/m³

The ultrastructural examination of the microwave exposed retinae revealed extensive changes both in synaptic terminals neuronal cell bodies and processes s well as in neuroglial cells A single exposure appeared to be sufficient to nduce similar although much less extensive changes Recently similar observa ions indicating damage of neurons in the brain were published by Albert & DeSantis (1975) and Switzer & Mitchell (1977) Hypothalamic neurons in brains

of adult hamsters displayed scarcity of rough endoplasmic reticulum and ribosomes as well as loss of microtubules In both these two studies the appearance of myelin figures in neuronal cells were frequently noticed as in retinal cells in the present study There were also signs of loss of neuronal in the exposed retinae Thus the results obtained in the present study concerning damage by microwave irradiation are in agreement with those previously published

We have also investigated the effect of 3100 MHz pulsed microwaves on microtubules and axonal transport (Paulsson et al 197-) There were no effects at power levels comparable to or higher than those in the present work The studies were restricted to acute exposures on *in vitro* systems and they may therefore not be applicable to the now observed retinal changes after repeated exposures

In the present work the brain received a microwave dose comparable to that of the retina In the barrier studies no significant leakage of Evans blue ... min through the blood brain barrier was observed even in animals with induced acute hypertension In contrast Frey et al (19 5) and Oscar & Hawkins (197) observed leakage of the blood brain barrier in microwave irradiated rats

The mechanisms underlying the observed changes in the retina are unknown The temperature in the orbit beneath the eye was found to rise to ... during the irradiation period and the intraocular temperature was found to be 40 C near the retina 3 min after the irradiation The action of some thermal effects is therefore likely to occur Whether non thermal effects are involved cannot be concluded

In conclusion the present work has demonstrated that ultrastructural changes occur in rabbit retina repeatedly exposed to 0.55 kW m 3100 MHz pulsed microwave radiation Further investigations should preferably be focused on the reversibility of these changes and the functional implications of these ultrastructural lesions

Acknowledgments

This work was supported by the Swedish Work Environment Fund and the Swedish ... Research Council (2-43) The authors thank E. Aurell B Tengroth and P L... for valuable discussions and A C Jacobsson and A M Karlsson for excellent technical assistance

References

ert E N & DeSantis M (1975) Do microwaves alter nervous system structure? Ann N Y Acad Sci 247 87–108
rell E & Tengroth B (1973) Lenticular and retinal changes secondary to microwave exposure Acta ophthal (Kbh) 51 764–771
mstrand C Johansson B & Rosengren B (1975) Blood brain barrier lesions in acute hypertensions in rabbits after unilateral X ray exposure of brain Acta Neuropath 31 97–109
ly L Jr Wakim K G Herrick J F & Parkhill E M (1948) Effects of microwave diathermy on the eye Amer J Physiol 15 432
y A H, Feld S R & Frey B (1975) Neural function and behaviour defining the relationship Ann N Y Acad Sci 247 433–439
ayson M C & Laties A M (1971) Ocular localization of sodium fluorescein Arch Ophthal (Chicago) 85 600–609
y A W Lin J C Kramar P O & Emery A F (1975) Effect of 2450 MHz radiation on the rabbit eye IEEE Trans Microwave Theor Tech 23 492–498
rsch F G (1970) Microwave cataracts – A case report reevaluated Electronic Product Radiation and the Health Physicist US Dept of Health Education and Welfare BRH/DEP 0–26 PB 195–742 pp 111–140
rnovsky N J (1965) A formaldehyde glutaraldehyde fixative of high osmolarity for use in electron microscopy J Cell Biol 27 137
kar K J & Hawkins T D (1977) Microwave alteration of the blood brain barrier system of rats Brain Research 126 (2) 281–293
ulsson L E (19 6) Measurements of 0 915 2 45 and 9 0 GHz absorption in the human eye In P deSantis Ed Conf Proc 6th Eur Microwave Conf pp 117–121 Microwave Exhibition and Publishers Ltd Sevenoaks
ulsson L E Hamnerius Y & McLean W G (1977) The effect of microwave radiation on microtubules and axonal transport Radiat Res 70 212–223
itzer W G & Mitchell D S (197) Long term effects of 2 45 GHz radiation on the ultrastructure of the cerebral cortex and on hematologic profiles of rats Radio Science 12 987–993

Author's address
Yngve Hamnerius
Research Laboratory of Electronics
Chalmers University of Technology
412 96 Goteborg
Sweden

The Department of Ophthalmology (Head B Ehinger)
and the Department of Histology (Head B Falck)
University of Lund Lund Sweden

INDOLEAMINE ACCUMULATING NEURONS IN THE RETINA OF CHICKEN AND PIGEON

A Comparison with the Dopaminergic Neurons

BY

I FLOREN

Recently a special group of indoleamine accumulating neurons has been described in the retina of some mammals and goldfish These neurons are characterized by their ability to accumulate indoleamines whereby they become visible in the fluorescence microscope They do not show any spontaneuos fluorescence The indoleamine accumulating neurons are in this study shown to be present in the retina of chicken and pigeon Their cell bodies differ from the earlier described cell bodies of the same type in other species in being larger and bottle shaped instead of round or oval and in being situated further outwards in the inner nuclear layer Their terminals ramify in three sublayers in the inner plexiform layer No indoleamine containing neurons could however be seen to fluoresce in normal retina of chick embryos newborn chicken older chicken or pigeons

Key words Indoleamine accumulating neurons – dopaminergic neurons
Retina – fluorescence microscopy – chicken – pigeon

Several of the so called biogenic monoamines (comprising catecholamines and indoleamines) are known or strongly suspected to be neurotransmitters in the brain namely adrenaline noradrenaline dopamine and 5 hydroxytryptamine Of these dopamine has been demonstrated in special neurons of the avian retina (Ehinger 1967 Stoeckel et al 1976) as in many other species and it has generally been presumed that this was the only monoaminergic neuron type

Received August 14 1978

retina However lately a new set of neurons has been found in the retina some species They are characterized by their ability to accumulate indol mines avidly and can therefore be suspected to use such a substance as their nsmitter They are also distinguishable on morphological grounds and have far been demonstrated in rabbit cat goldfish (Ehinger & Floren 1976) river nprey (Ehinger et al 1977) and Cebus monkey (Dowling et al 1979) The n of the present study was to see if they are also present in the retina of ds and, if so to describe their morphology Special attention was paid to npare them with the dopaminergic neurons (which also have an uptake me anism for biogenic monoamines and which could therefore be confused with indoleamine accumulating ones) Chicken were of particular interest be use of a previous report of an indoleamine containing cell type appearing in normal retina of chick embryos (Hauschild & Laties 1973) This special cell e was also searched for in the normal retina (i e without prior accumulation exogenously applied indoleamines) of chick embryos as well as in that of ler chicken

Materials and Methods

ick embryos (White Leghorn) 13 16 and 19 days newborn chicken (White Leghorn) icken (Derko a hybrid between Rhode Island and White Plymouthrock) 6–11 weeks d adult wild pigeons (Columba livia) were used

α methylnoradrenaline HCl (Hoechst Laboratories) 5 hydroxytryptamine 6 hydroxy yptamine (Sigma Chemical Co) and 5 6 dihydroxytryptamine (Regis Chemical Co)

Table 1
Treatment scheme and number of eyes

Treatment	Chick embryos	Newborn chicken	Chicken 6–11 weeks	Pigeons adult
lormal	10	9	20	4
μg α methylnoradrenaline	–	2	10	4
0–20 μg α methylnoradrenaline	–	2	5	4
5–20 μg 5 hydroxytryptamine 6 hydroxytryptamine or 5 6 dihydroxytryptamine*	–	2	26	6

On a molar basis 1 μg α methylnoradrenaline equals 2 μg 5 hydroxytryptamine 6 hydroxytryptamine or 5 6 dihydroxytryptamine

all three obtained as creatinine sulphate were dissolved in 20 μl 0.9% NaCl, ascorbic acid added (1 mg/ml) as antioxidant and injected intravitreally accord- the treatment scheme given in Table I. Niamid® (Sigma Chemical Co.), a mono- oxidase inhibitor, was given intraperitoneally in a dose of 100 mg/kg in two d... The animals were killed by decapitating which in older animals was preceded by e anaestesia.

Three or four pieces of retina were taken at random four h after the in- or intraperitoneal injection of the drug and were processed for fluorescence m... of *indoleamines and catecholamines according to the method of Falck and H...* In short the procedure involves freeze drying of the tissues, exposing them to f- aldehyde of controlled humidity and temperature whereby the fluorophores are for- paraffin wax embedding and sectioning. Catecholamines are in the microscope c... acterized by their greenish and indoleamines by their yellowish fluorescence (filter settings most often used in the fluorescence miscoscope, blue is the true f- escence colour of the fluorophore formed by catecholamines. The true colour of indoleamine fluorophore is yellow). For details of the histochemical procedure see, Falck & Owman ([illegible]4) or Björklund et al. (19[illegible]2).

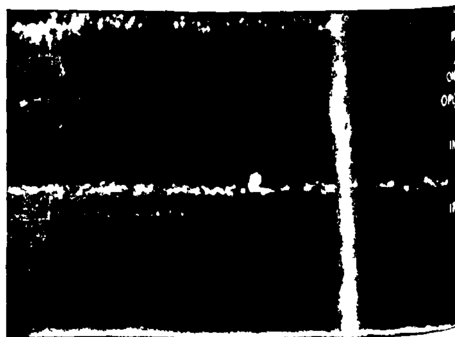


Fig. 1

Normal retina from an adult pigeon, fluorescence micrograph. There is one fluoresc- cell body in the innermost part of the inner nuclear layer and three sublayers of fluorescent terminals in the inner plexiform layer. The innermost sublayer is ver- sparse. Ph: photoreceptors; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer. × 330

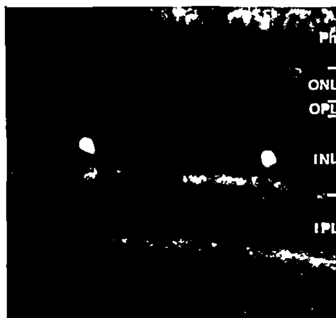


Fig 2

ina from an 11 week old chicken four h after the intravitreal injection of 20 μg dihydroxytryptamine Two bottle shaped cell bodies have appeared further out in the inner nuclear layer The innermost sublayer of fluorescent terminals is now almost as prominent as the outermost sublayer Fluorescence micrograph Designation of layer as in Fig 1 × 470

Results

l retina

e normal retina of chick embryos newborn chicken older chicken and treated according to the method of Falck and Hillarp contains flour cent *green round or slightly oval catecholamine containing perikarya in the* cell row of the inner nuclear layer among the amacrine cells (Fig 1) hey are the previously described dopaminergic neurons Processes from these ells were not as readily detectable in the chick embryos as in the older chicken nd the pigeons where they form three sublayers in the inner plexiform layers ne at its outer border one in its middle and one at its inner border (Fig 1) The outermost layer is most developed and the outer two are sparse The in termost layer is in fact often hard to discern

No yellow indoleamine containing cells can be seen with certainty in ... animals Treating with Niamid® a monoamine oxidase inhibitor for ... intraperitoneally (100 mg/kg) enhances the catecholamine fluorescence b... additional structure becomes visible

Retinas after the intravitreal injection of a catechollamine

Several attempts to inject substances into the vitreous of chick embryos w... made but they survived for less than one h and results with drug injectio... chick embryos are therefore not available

If small amounts (2 μg) of a catecholamine (α methylnoradrenaline) are i... jected into the vitreous of newborn chicken and older chicken a d p... four h before processing the retina there is a slightly enhanced green fl... scence of the structures described in the normal retina Sometimes a ...



Fig 3
Retina from an adult pigeon four h after the intravitreal injection of 90 μg 5,... dihydroxytryptamine Two bottle shaped cell bodies have appeared in the inner nucle... layer and the innermost sublayer of fluorescent terminals has increased to almost th... same density as the outermost sublayer Designation of layers as in Fig 1
Fluorescence micrograph × 400

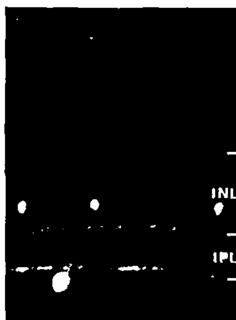


Fig 4

ina from a 6 week old chicken four h after the intravitreal injection of ?0 μg dihydroxytryptamine Besides three bottle shaped cell bodies in the inner nuclear :r a fluorescent cell body has also appeared among the ganglion cells Designation of layers as in Fig 1 Fluorescence micrograph × 400

e of bottle shaped cell bodies situated two or three cell rows outwards in inner nuclear layer were suspected

With higher doses of the catecholamine (10–20 μg) the bottle shaped cell lies appeared clearly They were seen to send processes to three sublayers the inner plexiform layer but now the innermost sublayer had increased l was about as pronounced as the outermost one

With this higher dose (10–20 μg) numerous bipolar like cells also appear l further outwards in the inner nuclear layer much like that seen with higher ses of an indoleamine Their cell bodies are oval or round and smaller than bottle shaped cell bodies and thereby readily distinguishable (cf Fig 5) eir processes reach both the outer and inner plexiform layers These bipolar e neurons are considerably more prominent in newborn chicken than in older cken and pigeons where they are sometimes hardly demonstrable

inas after the intravitreal injection of an indoleamine

jecting low to moderate doses (5–20 μg) of an indoleamine (5 hydroxy

tryptamine 6 hydroxytryptamine or 5 6 dihydroxytryptamine) intravitreal newborn chicken older chicken and pigeons results in yellowish fluore after four h in perikarya in the intermost part of the inner nuclear layer the lower doses however these perikarya which are the dopaminergic

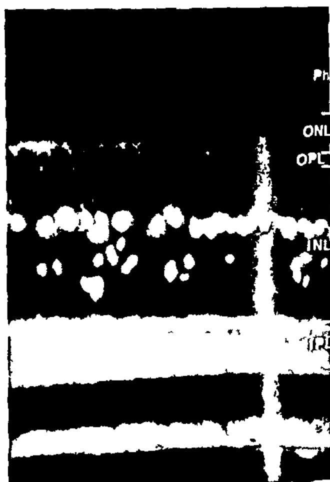


Fig 5
Retina from a newborn chicken four h after the intravitreal injection of ... 5 6 dihydroxytryptamine The same picture was seen with 10–70 µg α methylnoradrenaline The bottle shaped cell bodies are seen in the inner half of the inner nuclear layer Their processes reach the innermost sublayer A number of bipolar like cell bodies are seen in the outer half of the inner nuclear layer Their processes extend outwards as well as inwards In order to show these processes as well as possible other details appear t be less distinct Designation of layers as in Fig 1
Fluorescence micrograph × 410

re still of a more greenish colour The number of the cell bodies in the inner ost part of the inner nuclear layer is not increased as compared to the normal ina Two or three cell rows outwards in the inner nuclear layer another group neurons appear (Figs 2 and 3) They always displayed strong yellow fluo cence The number of these neurons is two or three times as high as that of dopaminergic neurons Their cell bodies are bottle shaped in contrast to round or oval dopaminergic ones and have a considerably larger size No nificant difference in the appearance or number of the bottle shaped cells re seen between central and peripheral parts of the retina Sometimes a few llowish fluorescing cell bodies were seen in the ganglion cell layer (Fig 4) e processes of all these cells ramify in three sublayers in the inner plexiform er The outer and innermost sublayers are now about equally developed igs 2 and 3) The middle sublayer is poorly developed and therefore does t show up in micrographs

Using the above amount (5-20 μg) of an indoleamine also reveals a great mber of yellowish fluorescing perikarya still further outwards in the inner clear layer with the same position number and appearance as those of the ove mentioned bipolar like cells also appearing after catecholamine treat nt (Fig 5) Especially with the lower indoleamine amounts (5 μg) the bottle aped cell bodies have a much more intense yellow fluorescence than those the bipolar like cells which with this dose are only faintly discernible In asing the injection amounts increases the fluorescence of the bipolar like lls but without reaching the same intensity as the bottle shaped cells

With indole doses higher than 25 μg fluorescence appears in all parts of e retina This is apparently an overdosage effect

Discussion

he occurrence of dopaminergic neurons in the retina is known in many species cluding chicken and pigeons (for reviews see Stell 1972 Graham 1973 Ro eck 1973 Ehinger 1976 1979) The neurons which appear green in the fluo scence microscope all have their cell bodies in the innermost part of the ner nuclear layer among the amacrines They send their processes to one r more sublayers in the inner plexiform layer In chicken and pigeons the utermost sublayer is best developed as confirmed in this study

A different group of indoleamine accumulating neurons which have their ell bodies at essentially the same position as the dopaminergic ones but with different distribution of their processes has been found lately (Ehinger & loren 1976) They are characterized by their ability to accumulate indoleamines

whereby they appear yellow in the fluorescence microscope It could be ar that the reason why more cells appear after injection of an indoleamine is that there is a second type of amine accumulating neurons present in the re Instead it could be suspected that the previously known dopaminergic ne may not always all be visible in normal animals but are made so when f to accumulate an indoleamine However the comparison between diff doses of a catecholamine and an indoleamine excludes this possibility be a low dose of the catecholamine will not label the indoleamine accumul neurons whereas a comparable dose of the indoleamine will label them e clusively Only with high doses will the substances enter both neuron t The observations which are made in this study agree with previous fi in the rabbit and goldfish retina

The morphology of the indoleamine accumulating cell bodies is quite u in the species investigated so far but the spread of the processes has been variable Thus rabbit and cat have round or oval cell bodies of approxim the same size as the dopaminergic ones They are situated at the junct the inner nuclear layer and the inner plexiform layer In goldfish t c bodies are somewhat smaller and often situated slightly more peripherall the inner nuclear layer than the dopaminergic ones The sublayering ca best developed either in the outer (goldfish) or inner part (rabbit) of the plexiform layer or be diffuse as in cat (Ehinger & Floren 1976) In the pre study which also reveals indoleamine accumulating neurons in chicken pigeons they are however found to deviate from the earlier known T their cell bodies are situated further outwards in the inner nuclear layer T are also considerably larger than earlier seen and bottle shaped with their n looking inwards extending in processes forming three sublayers The oute and innermost sublayers are about equally developed with a faint sublaye between This variation between different species in the morphology of t indoleamine accumulating neurons is not surprising if comparison is made the dopaminergic retinal neurons which show considerable variations not e in the distribution of their processes but also in the type of synaptic con established in different animals (Dowling & Ehinger 1975 1978 Dowling al 1979)

Ramon y Cajal in his classical studies described several types of amacr in the chicken retina (Ramon y Cajal 1972) but none is in very good agree with the fluorescence microscopical picture For instance the previously wel known dopaminergic neurons in the retina do not correspond to any s type of amacrines (Ehinger 1966) The same seems to be true for the ind amine accumulating neurons Among silver stained stratified amacrines o seems to fulfill very well the requirements of both being situated further o

rds in the inner nuclear layer and ramifying in three sublayers of the inner xiform layer as the indoleamine accumulating neurons do

Hauschild & Laties (1973) reported a spontaneously occurring indoleamine itaining cell in the retina of chicken embryos from the 14th day This cell newhat smaller than the dopaminergic one was observed in the inner nuclear er three of four cell rows from the inner plexiform – inner nuclear layer iction The relationship between this cell and the indoleamine accumulating ttle shaped cell now described seems close The reason why we have not been le to demonstrate any indoleamine fluorescence in the normal retina of chick ibryos or older chicken but only after the injection of amines is on the ier hand not clear It is however noteworthy that the cell found by Hau iild & Laties (1973) was observable only during a brief period of the devel ment It is possible that particularly favourable conditions with higher in leamine concentration might occur during a certain stage of development in breed of chicken used by Hauschild & Laties Some support for this is found the reports of rapid fluctuations in the activity of enzymes involved in the doleamine metabolism and also in indoleamine content in the eye of em yonic and newborn animals Thus Baker & Quay (1969) in the developing e of the frog Xenopus at hatching found a decreasing and then an increasing tivity of the enzyme forming 5 hydroxytryptamine 5 hydrotryptophan de rboxylase (5 HTPD) In the whole mouse eye the activity of 5 HTPD and AO (monoamine oxidase) rises in the middle of the first week post partum id then decreases (Smith 1973) 5 hydroxytryptamine and its metabolite 5 droxyindoleacetic acid (5 HIAA) are highest in the whole mouse eye the rst day post partum and then decline rapidly in the course of a couple of iys (Smith & Baker 1974) In chicken the MAO activity towards 5 hydroxy yptamine is low at the 12th day of incubation and then increases rebarkably itil the 18th day of incubation Thereafter the activity decreases and 2 weeks ter hatching the level is the same as measured in the adult (Suzuki et al 977b) Further in quantitative studies we have measured a higher amount of hydroxytryptamine in the retina of newborn chicken than in chicken of 11 eeks (Floren & Hansson 1978) The difference is statistically significant using tudent s *t* test Suzuki et al (1977a) reported even higher values in older nimals but their figures are not directly comparable because their method of nalysis was different

When higher amounts of an indoleamine are injected another population of eurons than the bottle shaped ones appears in the outer part of the inner uclear layer Their size is smaller and their gracile processes extend both utwards and inwards in the retina These characteristics are strongly sug estive of bipolar cells although the cell bodies of the supportive glial or Mul

lerian cells are located approximately at the same level of the retina. ... cesses of the Mullerian cells are however coarse in contrast to the ... processes of the bipolar cells and the processes of the Mullerian cells tra... almost the entire retina which neither the presently observed processes nor processes of the bipolar cells do When using lower amounts of an indolea... the bipolar like cells are hardly visible whereas the bottle shaped cells are very prominent Using high amounts of a catecholamine also makes the bip... like cells appear in chicken but only to a minimal extent in pigeon. In chicken the fluorescence (i e the amine uptake) of the bipolar like cells ... much stronger in newborn chicken than in chicken of 11 weeks The ... into identical bipolar like cells of either an indoleamine or a catecho... when used in high dose therefore seems to represent an uptake not necess... related to any transmitter function of the accumulated substances The f... that the uptake is higher in newborn than in older animals suggests an higher uptake into less differentiated bipolar like cells later undergo... cialization

The present demonstration of indoleamine accumulating neurons in birds to... gether with previous observations of apparently homologous neurons in ma... mals (Ehinger & Florén 1976 Dowling et al 1979) goldfish (Ehin... er & Fl... 1976) and river lamprey (Ehinger et al 1977) suggest their widespread oc... rence in the retina of vertebrates The transmitter of the indoleamine accu... lating neurons is however not known The only indoleamine which is curr... regarded as a neurotransmitter in the vertebrate brain is 5 hydroxytryptam... which can be seen in the fluorescence microscope However we have n... be able to demonstrate any neurons with the typical 5 hydroxytryptamine fl... scence in normal bird retinas Similar observations have been made in mam... (Floren 1978) The reason for this could be a very low concentration of hydroxytryptamine in the neurons but this seems somewhat unlikely bec... in other parts of the brain the 5 hydroxytryptamine neurons have suffici... transmitter concentration to make them demonstrable with the fluoresce... microscope Alternatively then the transmitter could be an indole other th... 5 hydroxytryptamine and incapable of forming fluorophores with formal... hyde in the Falck and Hillarp histofluorescence method There is indirect ev... dence that this may be the case in rabbits which will be discussed elsewher... (Florén 1978) and possibly this is also the case in chicken and pigeons.

Acknowledgments

This work was supported by grants from the A Wibergs Foundation the Magn Bergwalls Foundation the Faculty of Medicine in Lund and the Swedish Medical Research Council (project 04X-2321)

References

;
ier P C & Quay W B (1969) 5 hydroxytryptamine metabolism in early embryo enesis and the development of brain and retinal tissues *Brain Res 12* 2/3–29>

rklund A Falck B & Owman Ch (19/2) Fluorescence microscopic and micro pectro fluorometric techniques for the cellular localization and characterization of nogenic amines In Rall J E and Kopin I Eds *Methods of Investigative and liagnostic endocrinology* pp 318–368 North Holland Publ Corp

wling J E & Ehinger B (19/5) Synaptic organization of the amine containing in erplexiform cells of the goldfish and Cebus monkey retinas *Science 188* 2/0–2/3

wling J E & Ehinger B (19/8) Synaptic contacts of dopaminergic neurons in the abbit retina *J comp Neurol 180* 203–220

wling J E Ehinger B & Floren I (19 9) Fluorescence and electron microscopical observations on the amine accumulating neurons of the Cebus monkey retina In preparation

inger B (1966) Adrenergic retinal neurons *Z Zellforsch 71* 146–15_

inger B (196) Adrenergic nerves in the avian eye and ciliary ganglion *Z Zellforsch 8?* 5//–588

in er B (19/6) Biogenic monoamines as transmitters in the retina In Bonting S L, Ed *Transmitters in the Visual Process* Pergamon Press Ltd Oxford New York

inger B (19 9) Biogenic monoamines and amino acids as retinal neurotransmitters In Cool S J and Smith E Eds *Frontiers in Visual Science* Springer Series in Optical Sciences Springer Verlag Berlin Heidelberg New York (in press)

inger B & Floren I (19/6) Indoleamine accumulating neurons in the retina of rabbit cat and goldfish *Cell Tissue Res 1/5* 3/–48

inger B, Holmberg K & Öhman P (19/7) Aminergic and indoleamine accumulating neurons in the retina of the river lamprey (Lampetra Fluviatilis) *Acta ool (Stockholm) 58* 117–1?3

lck B & Owman Ch (1965) A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic amines *Acta Univ Lund Sectio II No 7* 1–23

oren I (19/8) Arguments against 5 hydroxytryptamine as neurotransmitter in rabbit retina *J Neurol Transm* (in press)

oren I & Hansson H C (19 8) Aspects on 5 hydroxytryptamine as a neurotransmitter in the retina of rabbit and chicken *Invest Ophthalmol* (in press)

raham L T (1973) Comparative aspects of neurotransmitters in the retina In Dawson H and Graham L T Eds *The Eye Vol 6* pp ?83–34? Academic Press

auschild D & Laties A M (1973) An indoleamine containing cell in chick retina *Invest Ophthal 1?* 537 540

amon y Cajal S (1972) *The Structure of the Retina* Compiled and translated by S A Thorpe and M Glickstein Charles C Thomas Publ Springfield Illinois

od eck R N (1973) *The vertebrate retina* Freeman San Francisco

mith M D (1973) 5 hydroxytryptophan decarboxylase (5 HTPD) and monoamine oxidase (MAO) in the maturing mouse eye *Comp gen Pharmacol 4* 175–178

mith M D & Baker P C (1974) The maturation of indoleamine metabolism in the lateral eye of the mouse *Comp biochem Physiol 49 A* 281–286

pbth I 5 2 14

Stell W K (1972) The morphological organization of the vertebrate retina In F M G F Ed *Physiology of photoreceptor organs* Vol VII/2 pp 11? ?13 Berlin Heidelberg New York
Stoeckel M L Roussel C Zwiller J Madarasz B & Porte A (19/6) Concen... of dopaminergic fibres in the marginal zone of the bird retina A nonvisual p... receptor system? *Cell Tissue Res* 1/3 335–341
Suzuki O Noguchi E Miyake S & Yagi K (19/7a) Occurrence of 5 hydroxytr... mine in chick retina *Experientia* 33 9?7–9?8
Suzuki O Noguchi E & Yagi K (1977b) Monoamine oxidase in developing c... retina *Brain Res* 13? 30?–313

Author s address

Ingrid Florén
Department of Ophthalmology
University Hospital
S ?21 85 Lund
Sweden

Department of Ophthalmology (Head Thore Lie Thomassen)
Rikshospitalet The National Hospital University of Oslo Oslo

TYPICAL AND ATYPICAL MONOCHROMACY STUDIED BY SPECIFIC QUANTITATIVE PERIMETRY

BY

EGILL HANSEN

Two blue cone monochromats and four rod monochromats have been studied by increment threshold measurements applying the Stiles principle Some rudimentary colour discrimination was reported by the blue cone monochromats One patient showed good discrimination between short and middle wavelength lights in matching experiments using the Nagel II apparatus His neutral band in the spectrum was at λ = 483–493 nm Dichromatic vision could not be proved in the other patient
The blue cone monochromats also had good responding blue mechanism in the periphery Indication of cone activity other than blue cones is found in both kinds of monochromats these cones being probably of the rhodopsin cone type (π_0 cones) The conclusions are drawn that the inhibitory effect of the π cones upon the rod mechanism may account for the differences shown by our two blue cone monochromats as to visual acuity nystagmus and photophobia Likewise their differences regarding dichromatic vision may be explained by an unequal number of π cones in their retinas rather than by differences in their blue mechanisms

Key words blue cone monochromacy – rod monochromacy – colour vision – static perimetry – Stiles functions

An atypical form of monochromacy designated blue mono cone or blue cone monochromacy was first described by Blackwell & Blackwell in 1957 It differs from the typical rod monochromacy by the presence of functioning blue cones in addition to rod receptors The condition is very infrequent In a review by Alpern (1974) only 20 cases all males had been verified By ordinary

Received September 6 1978

methods including the greater part of the colour vision tests a d a- scopic examinations there may not be any characteristic differences the typical and atypical monochromacies

Perimetric registrations during selective chromatic adaptation (Ha se make it possible to study the receptor mechanisms separately by the ce and peripheric response functions It is the purpose of this s udy t re characteristic findings in persons who are blue cone monochromats a d comparisons with the findings in typical total monochromats who ha examined with the same methods

Material

Case 1 (BTW) a 1" year old male is a blue cone monochromat. There w consanguinity in the family and no other cases of amblyopia or c l defects He is the second child The visual impairment had been prese birth The patient had moderate photophobia in daylight He ma a in twilight He confused yellow and green which however were d s from blue Blue violet and purple were confused like red black and He exhibited a coarse pendular nystagmus Fixation was central Haidinger s brushes could not be seen Normal pupillary reactions were On ophthalmoscopy the fundus appeared normal including the op ic and retinal vessels Visual acuity was 6 60 on each eye Normal visual limits were found in the Goldmann perimeter (Object si e 16 mm 10 intensity) His dark adaptation curve obtained in the Goldmann Wee adaptometer was subnormal monophasic (final thresholds 1'4 log u above normal level)

Case 2 (HH) a 1 year old male is a blue cone monochromat. Th p always been partially sighted A heavy jaundice had been noticed after He was in good health There was no consanguinity in the family and no cases of amblyopia He is the only child The parents have been found by colour vision tests His maternal grandfather a protan pe was ex here A brother of the grandfather is also said to be red green deficie patient had no photophobia and performed well in twilight There was a s inconstant nystagmus The pupillary reactions were normal Ophthalmo showed a normal fundus and a faint foveolar reflex Visual acuity was each eye Colour vision had always been poor Yellow his favorite colour easiest to recognize but could sometimes be confused with green He co also red and black Blue as a rule was not distinguished f om violet p purple On the other hand was not confused ow or green

The following four patients who were typical rod monochromats had not n able to distinguish colours They all had some photophobia *Case 3* (OF) 30 year old male who had moderate nystagmus like *Case 4* (ER) a female years of age *Case 5* (NN) a male 32 years of age who had very slight ny gmus *Case 6* (OK) a male 35 years of age in whom no nystagmus could be n Visual acuity which was optimal at reduced illumination was 5/36 in es 3 and 6 6/20 in case 5 and 5/60 in case 4 They all had slight to mode e hypermetropia and a moderate regular astigmatism (cases 4 5 and 6) w pupillary reactions were noticed (cases 3 5 and 6) On ophthalmoscopy rmal optic discs were found as well as normally calibrated vessels In cases nd 5 the macula was poorly marked Foveolar reflexes were not seen ERG istered in patient 6 showed a normal response to single flash stimuli under otopic conditions but no response to flicker at 25 Hz The father as well as ir brothers of patient 3 were rod monochromats The parents were first usins Rod monochromacy was found in a brother and a sister of patient 5

Methods

ie colour vision tests used comprise the Ishihara test (11th ed) the AO HRR test Farnsworth s D 15 test the Farnsworth Munsell 100 Hue test the Farnsworth s tan plate and the Sloan s achromatopsia test A series of charts reproduced in eyish shades from charts 10–17 of the Ishihara test (Hansen 1963) were shown rallel with the pseudo isochromatic tests The pigmentary tests were administered der Macbeth Illuminant C lamps at about 300 lux

Anomaloscopic examinations were performed with a Nagel anomaloscope type I dditional examinations were performed in the blue cone monochromats with a cali ated Nagel anomaloscope type II where colour equations could be tested also in e short wavelength part of the sp ctrum The widest aperture of the apparatus was ed (3 12 visual angle)

Static perimetry under varying adapting conditions was performed in a modified oldmann perimeter as described earlier (Hansen 19 4 Hansen & Seim 19 8) Near ionochromatic interference filters (half band width 10–15 nm) were used and in some speriments *moderately narrow banded interference filters (half band width 30–47* m) The angular size of the target is expressed in degrees and minutes As the target lightly eleptical the diameter of the equivalent circular target with the same area indicated The background illumination was measured with a luxmeter (Hartmann & rown EBLX3) in the bottom of the bowl The luminance of the test targets was cal ulated as the reflected energy at the level of the sphere Fixation could be controlled y means of the telescope of the apparatus except for registrations done in total dark ess In cases of obvious nystagmus fixation was unstable making the statement of erimetric angles only approximate

Results

Pigmentary tests

The pseudo isochromatic tests could not be read by any of the rod monochromats except for some cautral charts The blue cone monochromats could see hidden digits and the transformation pattern charts (mainly the red green alternative) of the Ishihara tests At the AO HRR test patient 1 could see three of the blue yellow charts and one of the red green charts Patient 2 could see all the blue yellow charts but one and four of the red green charts A series of charts reproduced in greyish shades from the Ishihara test could be seen by the rod monochromats as well as by the blue cone monochromats With Sloan's achromatopsia test a typical achromatic pattern was obtained for the rod monochromats and for patient 1 who could not however make a grey match with the yellow print Patient 2 matched a dark grey (33) with the red print but did not make matches with the other colours At the Farnsworth's tritan plate the blue cone monochromats could see the green square and not the blue square whilst two rod monochromats were unable to see either square Several performances with the Farnsworth's D 15 test revealed confusions without exact protan or deutan axis but mainly in the red green range for the blue cone monochromats whilst the rod monochromats demonstrated a typical scotopic pattern (Fig 1) Patient 2 had 244 error scores at the 100-Hue test The pattern was moderately distorted with the least errors in the yellow red and in the blue to blue green regions (a reversed tritan pattern

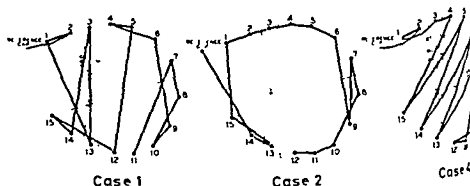


Fig 1

Performance of two blue cone monochromats (Case 1 and 2) on the Farnsworth's D 15 test compared with a typical performance of a rod monochromat (Case 4)

Table I

e performance in the Nagel anomaloscope type I of two blue cone monochromats se 1-2) and 4 rod monochromats (case 3-6) The figures indicate the yellow values used to match the upper field (the red green values)

	Red green value						
Case	73	60	55	50	40	30	0
1	½-1		34½		7/		Not possible
2	½-1	25		43½	61	9_	Not possible
3	1-1½				55		Not possible
4	1				54		Not possible
5	½-1	25			56		Not possible
6	1				61½		Not possible

nomaloscope tests

With the Nagel anomaloscope type I a typical achromatic pattern was found all the patients (Table 1) Matches all over the scale were accepted except r the green values which were too bright for matching with the yellow field ed was matched with a very dark yellow

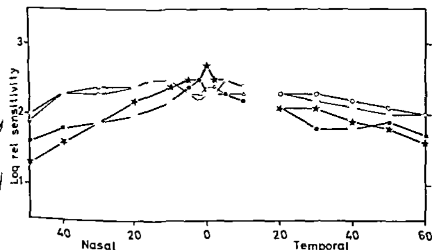


Fig 2

Static perimetry in white background (10 cd/m²) for two blue cone monochromats (● BTW ★ = HH) and two rod monochromats (○ = OF △ = ER) Angular diameter of target is 54 Shaded area indicates the normal variation (mean ± 2 SD)

With the Nagel anomaloscope type II patient 1 showed an uncertain spectral hue in the region 480–505 nm while he indicated the sensation of colour for the shorter wavelength values and yellow for the longer wavelength values However for a series of fixed settings of the main screw he accepted matches for wavelengths varying between 4.3 and 566 nm

Patient 2 indicated a neutral spectral range at λ = 485–495 nm while he the sensation of blue in the shorter wavelength region and yellow or green the longer wavelength region He could match the wavelengths 51? a d nm but did not accept any match for for the wavelengths 485 and 5?? or for the wavelengths 468 and 490 nm The cyan blue equation λ₁ + λ₂ was performed with a moderately small range of settings using the main screw (12 5–20 scale values)

Quantitative perimetry

The perimetric profiles obtained under standard conditions are shown in Fig *The curves are deflected and irregular in the central part for patient 1 (BTW* as well as for the two rod monochromats Patient 2 (HH) who had a better visual acuity has a normal central profile though at a reduced level T

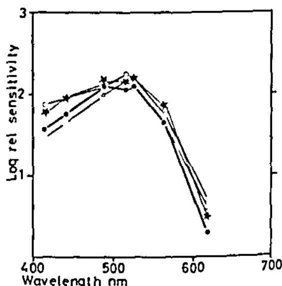


Fig 9
Relative spectral sensitivity in white background (10 cd m) registered by use of moderately narrow banded interference filters ● = BTW ★ = HH △ = OF ○ ER
Angular diameter of target 54 Shaded area indicates the normal variation (mean ± 2 SD)

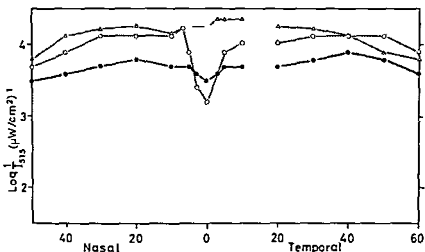


Fig. 4

Static perimetry during total dark adaptation recorded with green object light (λ_m - 515 nm) Ordinate absolute threshold sensitivity for target subtending 9' angular diameter ● = BTW o = OK △ = OF Shaded area indicates the variation (mean ± 2 SD) for 5 normals

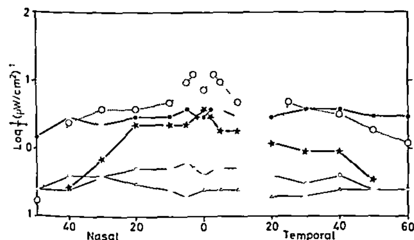


Fig. 5

atic perimetry in yellow adapting light (λ = 559 nm 9000 lux) registered with near onochromatic object lights Blue violet light (λ_m = 439 nm) is used in the registra on of BTW (●) HH (★) and the normal observer (stippled line) and green light m = 500 nm) in the registration of the rod monochromats OK (o) and OF (△) Angular diameter of target 1° 47

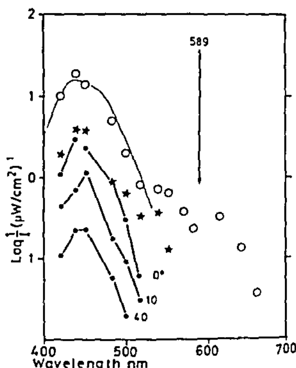


Fig 6

Spectral threshold sensitivity registered in BTW (●) HH (★) and a normal pers the same age group (o) under conditions as in Fig 5 The records of BTW ar tained centrally and at the perimetric angles 10 and 40 in the nasal field, the b curves being displaced downwards and separated by ½ log unit The thin line indi the action spectrum of the blue mechanism (after Walraven 19)

perimetric curves of the blue cone monochromats are still below the no level in the periphery while those of the two rod monochromats reach normal level In the white background light (10 cd/m) the spectral sensit curves are quite identical for the two types of monochromats with a maxi sensitivity at about 500 nm (Fig 3) Static perimetry performed during t dark adaptation shows a quite flat perimetric curve for the blue cone m chromat case 1 as well as for the rod monochromat case 3 whilst the o rod monochromat case 6 has a perimetric profile which is well fitted to normal pattern (Fig 4)

In the bright yellow light the rod monochromats were not able to see short wavelength targets neither centrally nor peripherally However the ject lights at λ_m 500 nm were easily seen and could be followed tow

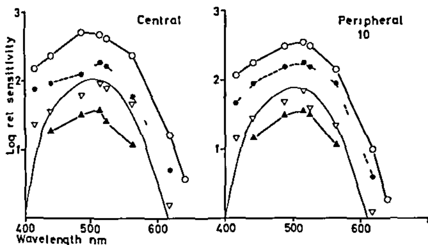


Fig 7

lative spectral sensitivity registered by use of moderately narrow banded interence filters in a rod monochromat (ER) in different adapting lights (from top to ttom) purple white yellow and blue A fitted scotopic luminosity curve (the C I E V_λ curve) is indicated by the thin line

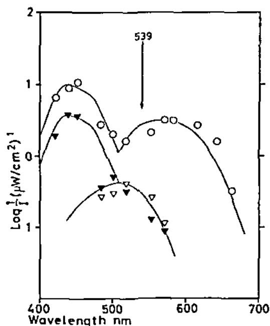


Fig 8

pectral threshold sensitivity in a near monochromatic green light (λ_m ~539 nm, 30 lux) Angular diameter of target 1 47 ▼ - BTW ▽ = NN Open circles indicate he response of a normal person Full lines indicate the blue and red mechanisms (after Walraven 1974) and the C I E scotopic luminosity curve (lowest)

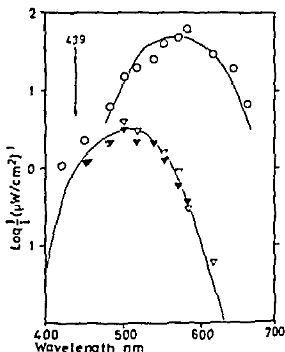


Fig 9
Spectral threshold sensitivity in a near monochromatic blue violet light (λ_m ... 439 ... 3 8 lux) Explanation as for Fig 8

the periphery (Fig 5) The curves for the rod monochromats expressing the peak spectral sensitivity are at low levels For the blue cone monochromats static perimetry with the object light at λ_n = 439 nm could be performed showing a clearly higher sensitivity even though the sensitivity level in general does not reach that of a normal person Spectral sensitivity curves registered under the same conditions are shown in Fig 6 The peak sensitivities coincide with that of the blue cone mechanism Patient 1 (BTW) has nearly identical response levels both centrally and at the perimetric angles 10 and 40

The typical rod monochromats show the same type of action spectrum irrespective of the quality of the background illumination This indicates that only one receptor mechanism is present and responsible for the sensation. In two of the rod monochromats case 3 and case 4 quite identical registrations were obtained centrally and at the 10 nasal position Fig 7 shows the results for one of the patients (case 4)

Fig 8 shows the action spectrum of case 1 one of the blue cone monochromats in a green background light as compared with the response of a

nochromat case 5 and a normal observer The curves for the blue cone nochromat indicate the presence of two kinds of receptors which are in ac dance with the π_1 and π_0 mechanisms of Stiles Only one type of receptor chanism is demonstrated in the rod monochromat With a blue violet back und light case 1 records an action spectrum which is in accordance with the mechanism and is identical with the response of the rod monochromat g 9)

Discussion

measurements in the standard luminance of the perimeter (10 cd/m^2) our blue ne monochromats show a typical rod response pattern the same as that of the d monochromats (Fig 3) Blackwell & Blackwell (1961) found bimodal curves th peaks corresponding to the blue cone and rod mechanisms and so did korny et al (1970) In spite of several attempts Alpern et al (1965) could t obtain a bimodal curve bu they found in their blue cone monochromat at the luminosity curve was dominated by a pure rod curve or by a pure blue eptor curve depending on the background illumination Registration of lu nosity curves in white backgrounds therefore may not be a reliable method separating the two types of monochromats

The luminosity curves registered against the yellow background of the peri ter (Fig 6) show a distinct blue receptor response in both our blue cone nochromats The peak sensitivity which approximately is at the same level r the two patients is somewhat below that of the normal observer The typical d monochromats being unable to see the blue violet target at all but exclu ely the 500 nm target have reduced sensitivity by at least 1 log unit in the ue spectral region in relation to the blue cone monochromats Therefore a dis mination between the typical rod monochromats and the blue cone mono romats can be obtained by perimetry using blue targets and a low pressure a lamp as the adapting light

Blackwell & Blackwell (1961) in their blue cone monochromats found blue ne response only in the central visual field and stated that there was no evi nce that a significant number of functioning blue cones exist at a retinal cation 15 from the fovea This is not in accordance with the present findings our patients The static perimetry curves obtained in bright yellow light in cate that there are also well functioning blue receptors in the periphery to e same extent as in normal individuals (Fig 5) Even with the gross nystag us displayed by patient 1 no response would be expected at the 40–60 peri etric angles if the blue cones were strictly limited to the central area In both ur blue cone monochromats the perimetric curves expressing the blue mechan

ism are somewhat reduced in relation to the curve of the normal obser- patient 2 with a good central fixation it is noteworthy that the threshold sensitivity is highest at the fixation point and not paracentrally which is typ[illegible] normal individuals This may indicate a blue cone distribution in the retina patient 2 that is different from normal persons or else a possible lack of [illegible] pigmentation

Blackwell & Blackwell (1961) suppose that in the mesopic zone where the two receptor systems are acting the blue cone monochromats are blue cone rod *dichromats* In the Nagel II anomaloscope patient 1 indicated a [illegible] neutral band in the spectrum but his dichromatic vision could not be con[illegible] by the matching experiments However in this case the examination was car- ried out with light of wavelengths only above 473 nm and his real neutral [illegible] might have been at shorter wavelengths Patient 2 more confidently des[illegible] a neutral band in the spectrum at λ = 485–495 nm and his dichromatic [illegible] was confirmed by the matching experiments In this patient the exact equation ([illegible]) was particularly good for proving dichromatic [illegible] in the short wavelength part of the spectrum the lower reference field be being coincident with his neutral point This equation in the Nagel II [illegible] ment which was originally intended for testing tritanopes has been aban[illegible] because of the different colour saturation in the two fields

In her study of achromats Sloan (1958) postulated the presence of ph[illegible] rods Alpern et al (1971) suppose that the rudimentary colour vision of blue cone monochromats is mediated by blue cones and π_0 receptors wh[illegible] not rods but *cones* They found that these receptors have the directional se[illegible] sitivity and the dark adaptation curve of normal red and green cones The cones are not necessarily present in all blue cone monochromats Daw & En[illegible] (1973) examined one patient and did not find evidence for cones h[illegible] 500 nm pigment π cones may be present in unequal numbers also in rod m[illegible] chromats For instance among our rod monochromats patients 6 (OK) d[illegible] no nystagmus and had a relatively good central vision which might indica[illegible] high number of π cones His nearly normal perimetric profile obtained [illegible] dark adaptation (Fig 4) may also indicate that his central receptors are of kind different to ordinary rods

Green (1972) found that the blue receptors in blue cone monochromats well as in normal individuals contribute very little to the visual acuity [illegible] more uncertain to which extent the π cones may contribute to visual acu[illegible] Possibly their influence on the visual acuity may be an indirect one due to inhibition of the π rods under photopic conditions This also might be [illegible] explanation for the differences in photophobia and nystagmus seen in m[illegible] chromats

Blue cone activity is considered to cause inhibition of the rod system A re ...ed recovery rate of the rod mechanism after exposure to bright yellow light ...s seen in patient 1 (BTW) (Hansen et al 19/8) Blackwell & Blackwell (1961) ...und photophobia gross nystagmus and low visual acuity in their class III ...ients, presumably having the poorest blue receptor function Grutzner (1964) ...led attention to his atypical monochromats who showed inhibition of rod ...sitivity under photopic conditions in spite of their loss of red and green ...nes Trezona (19/0) suppose that the inhibition of rods by cones is effected ...rticularly by the blue cones However the blue cones may not be the only ...nibitor of rod activity in blue cone monochromats

As our blue cone monochromats have good responding blue mechanisms as ...und by the chromatic adaptation studies and their threshold sensitivity is not ...rticularly different it is unlikely that their variance in visual acuity nystag ...us and photophobia is due to differences in their blue mechanisms What is ...ore likely is that the better performance of patient 2 under photopic condi ...ns as well as his better colour discrimination and clear dichromatic matches ...the short wavelength part of the spectrum is due to a higher number of π ...nes in his retina

References

...pern M Lee G B & Spivey B E (1965) π_1 cone monochromatism *Arch Ophthal (Chicago) 74* 334–337

...pern M Lee G B Maaseidvaag F & Miller S S (19/1) Colour vision in blue cone monochromacy *J Physiol (Lond) 21?* 211–233

...pern M (19/4) What is it that confines in a world without color? *Invest Ophthal 13* 648–674

...ackwell H R & Blackwell O M (19?) Blue mono cone monochromacy A new color vision defect (Abstract) *J opt Soc Amer 4/* 338

...ackwell H R & Blackwell O M (1961) Rod and cone receptor mechanisms in typical and atypical congenital achromatopsia *Vision Res 1* 6?–107

...aw N W & Enoch J M (19/3) Contrast sensitivity Westheimer function and Stiles Crawford effect in a blue cone monochromat *Vision Res 13* 1669–1680

...reen D G (19/2) Visual acuity in the blue cone monochromat *J Physiol (Lond) ???* 419–4?6

...rutzner P (1964) Der normale Farbensinn und seine Abweichungen In *Deutsche ophthalmolo ische Gesellschaft Bericht 66* 161–1 2

...ansen E (1963) Factors causing uncertainty when conducting colour discrimination tests *An Inst Barraquer (Barcelona) 4* 2?0–?9?

...ansen E (1974) The colour receptors studied by increment threshold measurements during chromatic adaptation in the Goldmann perimeter *Acta ophthal (Kbh) 5?* 490–500

Hansen E & Seim T (1978) Calibration of the Goldmann perimeter and accessories used in specific quantitative perimetry. Acta ophthal (Kbh) 56 241-251
Hansen E, Seim T & Olsen B T (1978) Transient tritanopia experiment in blue monochromacy. Nature (Lond) 276 390-391
Pokorny J, Smith V C & Swartley R (1970) Threshold measurements of spectral sensitivity in a blue monocone monochromat. Invest Ophthal 9 807-813
Sloan L L (1958) The photopic retinal receptors of the typical achromat. Am J Ophthal 46 81-86
Trezona P W (1970) Rod participation in the 'blue' mechanism and its effect on colour matching. Vision Res 10 317-332
Walraven P L (1974) A closer look at the tritanopic convergence point. Vision Res 14 1339-1343

Author's address

Egill Hansen
Department of Ophthalmology
Rikshospitalet, The National Hospital
University of Oslo
Oslo
Norway

Department of Pharmacology (Head Bengt Ablad)
AB Hassle Molndal Sweden[1])
and Department of Pharmacology (Head Lennart Lundholm)
University of Linkoping Linkoping Sweden)

INHIBITION AND FACILITATION OF LACRIMAL FLOW BY β ADRENERGIC DRUGS

BY

G ABERG[1]) G ADLER[1]) and J WIKBERG)

The effects of adrenergic β stimulating and β blocking compounds on tear flow were studied in conscious rabbits with a modified Schirmer technique The tear flow was significantly increased by the unselective β adrenergic agonist isoprenaline and by the selective β_1 agonist H 80/62 (racemic Prenalterol Hassle Sweden) The effects of the agonists were blocked by the unselective β adrenergic antagonist propranolol (Inderal®) and by the selective β_1 antagonist metoprolol (Seloken® Lopressor®) The experimental data favoured the hypothesis that the β receptors involved in the regulation of the tear flow were of the β_1 type The anatomical location of these β receptors is at present unclear but *in vitro* experiments performed on the rabbit lacrimal glands indicated that the receptors were probably not located on cholinergic nerve endings as the release of ace tylcholine was not influenced by isoprenaline

Key words tear flow – adrenergic betablockers – adrenergic betastimula tors – animal experiments

a reduction in tear flow was found in patients with conjunctival changes r practolol (Eraldin® Eraldina®) (Wright 1978) it was of interest to study possible effects of other adrenergic β blocking agents – and especially β_1 :kers – on tear flow Earlier studies in animals (Botelho et al 1973 Boissier l 19/6) have indicated that stimulation of adrenergic receptors may increase lacrimal flow while adrenergic blocking agents have been reported to de

eived April 7 1978

crease the tear flow In this investigation we have tried to further elucid mechanism for the action of adrenergic β blocking agents on tear flow. determine which types of adrenergic β receptors that may be involved in mechanisms

Methods

I Experiments with conscious rabbits

The test of Schirmer (1903) and Norn (1974) was modified and a method according to Boissier et al (1976) was developed for measuring tear f conscious rabbits The rabbits were kept in ordinary laboratory rabbit cages their tear flow was measured with strips (3 × 40 mm) of filter paper (M OOR) A mark was made 5 mm from one end of the filter paper strip and the strip was applied to the rabbit's eye with the proximal 5 mm in the conjunctival sac and the remaining 35 mm hanging outside The tear fluid then taken up by the filter paper and after 60 sec the filter strip was out After a time lapse of another 5 sec during which tear fluid was descending along the strip a second mark was made on the strip at the b between the dry and the tear wetted area This procedure was repe every 5 min with new strips of paper for both eyes and the mean value of distances between the 5 mm ink mark and the second mark was assumed represent the tear flow

When the tear flow had been stable for 15 min saline or an adrenergic blocking agent was infused intravenously in a volume of 0 2 ml/min for 1 via a marginal vein of an ear Exactly 15 min after the termination of the f iv infusion Isoprenaline was given iv for 20 min via the same catheter dose of isoprenaline used was 0 3 μg/kg/min in a volume of 0 2 ml/min saline with ascorbic acid 0 1 mg/ml

In experiments when isoprenaline was replaced by the β_1 selective H 80 62 (racemic prenalterol Carlsson et al 197) the latter was given dose 60 μg/kg/min in 0 2 ml/min of saline for 3 min Thus the total dose H 80/62 used in the present experiments was 180 μg/kg

II Experiments with isolated rabbit lacrimal glands

Rabbits of either sex were killed by stunning and bleeding and the lacri glands were rapidly excised through the caudal supraorbital incisura and fr from its capsula The al gland was mounted in a small organ bath suspended in a Kreb solution containing (mmol/l) NaCl 1930 MgCl

1 2 5 $NaHCO_3$ 15 4 KH_2PO_4 1 2 glucose 5 5 and eserine sulphate 0 03 solution was kept at 37°C and aerated with a mixture of O (95 %) and (5 %)

he preparation was incubated with 1 0 μmol/l of [³H]choline (methyl]cholinechloride 10 0 μCi/ml) for 60 min and during that period it was inuously given electrical field stimulation with a supramaximal voltage msec and 0 1 Hz from two transversally mounted platinum electrodes After incubation in [³H]choline the preparation was washed by superfusion with h Krebs solution for 30 min Then 4 ml samples of the superfusate were n every 5 min during the whole experiment for analysis of [³H]choline and]acetylcholine according to Wikberg (1977) The [³H]acetylcholine was also itified by ion exchange chromatography according to a double isotope tech le described by Wikberg (1979) At the end of each experiment the radio vity of the whole lacrimal gland was determined by dissolving it in 1 ml uene® Then 10 ml of a scintillation fluid containing 50 mg PPO (diphenyl zole) and 3 mg POPOP (2 2 p phenylene bis (5 phenyloxasole)) in toluene added and the radioactivity was measured in a Packard Tri Carb scin ation counter The counting efficiency was determined by an internal stan d and varied between 30–35 per cent for the different samples In the pre t experiments the release of [³H]choline and [³H]acetylcholine into the perfusate were calculated as per cent of the total radioactivity calculated to present in the tissue before the first fraction was collected (Wikberg 1977)

In order to study the effect of adrenergic β stimulation on the release of tylcholine isoprenaline was added to the superfusing Krebs solution both electrically stimulated and to unstimulated tissues

Mean values standard error of mean values and Student s *t* test were cal lated on a computer using standard formulas

Results

Experiments with conscious rabbits

ffects of isoprenaline on basal tear flow In some preliminary experiments it as found that isoprenaline 0 3 μg/kg/min infused intravenously in 0 2 ml of aline/min increased the tear flow by maximally 25 ± 3 per cent (N = 5) The increase in tear flow was fairly stable for at least an infusion period of 20 in Isoprenaline 0 3 μg/kg min increased the heart rate by maximally 115 ± 10 eats/min (N = 4) as measured from the ECG The effects of isoprenaline 0 3

µg/kg/min were submaximal as isoprenaline 1 0 µg/kg/min caused an incr. in tear flow of maximally 51 ± 4 per cent (N = 4) and increased the heart r. by maximally 172 ± 16 beats/min (N = 4) (Fig 1)

Effects of β blocking agents on basal tear flow Neither propranolol (Inder in doses up to 6 0 mg/kg nor metoprolol (Seloken® Lopressor®) in dos. to 30 0 mg/kg had any significant effects on basal tear flow (Table I).

Effects of β blocking agents on isoprenaline induced increase of tear f Both propranolol and metoprolol decreased the isoprenaline induced i. of tear flow in a dose dependent manner (Figs 2 and 3) ED_{50} for the pro. nolol induced decrease of tear flow was approximately 1 0 mg kg The co. responding value for the β_1 selective blocker metoprolol was approx. 5 0 mg/kg (Fig 3)

Effects of H 80 62 on basal tear flow The basal tear flow was increased H 80 62 In dose finding experiments it was found that H 80 62 given i. venously in the dose 60 µg/kg/min for 3 min increased the basal tear f. with maximally 37 ± 7 per cent (N = 6) i e slightly more than a co. infusion of isoprenaline 0 3 µg/kg/min The dose of H 80 62 presently u. was a submaximal one as after H 80 62 300 µg kg/min for 3 min the b.

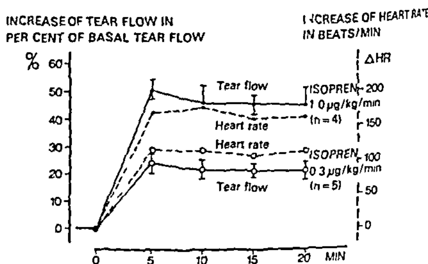


Fig 1

Effects of isoprenaline on basal tear flow and heart rate when given intravenously for 2 min in the doses 0 3 and 1 0 mg/kg/min to conscious rabbits For heart rate standard errors of the means are not shown for the sake of simplicity the s.e.m. were consistently less than 10 per cent of the mean values shown in the figure

Table I

...cts of β blockers on basal tear flow The basal tear flow 3 min before intravenous ...inistration of saline or a β blocker was called 100 per cent No statistically ...ificant changes in the basal tear flow were caused by the β blockers ($P > 0.05$)

β blocker	Dose (mg/kg)	N	Tear flow (%) at two different times after β blockade (mean ± SEM)	
			3	13
NaCl 0.93 %	–	15	100 ± 4	97 ± 3
Propranolol	1.0	6	107 ± 7	110 ± 7
"	3.0	4	99 ± 8	97 ± 11
	6.0	4	104 ± 8	99 ± 8
Metoprolol	3.0	9	99 ± 1	100 ± 4
	10.0	8	100 ± 1	100 ± 6
	30.0	4	100 ± 5	101 ± 5

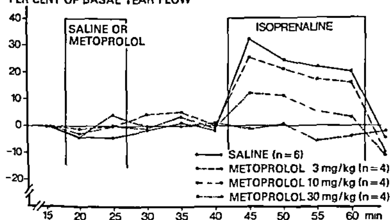


Fig 2

...fects of metoprolol 3 10 and 30 mg/kg on isoprenaline induced increase of tear ...w The SEM values are not shown for simplicity reasons During the infusion of isoprenaline SEM was less than 5 per cent of the basal tear flow

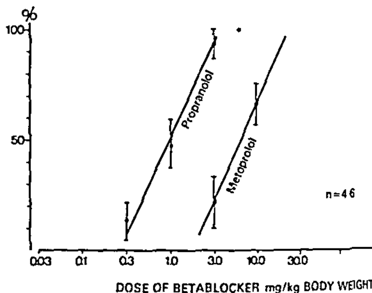


Fig 3

Inhibitory effects of metoprolol and propranolol on isoprenaline induced increase of tear flow

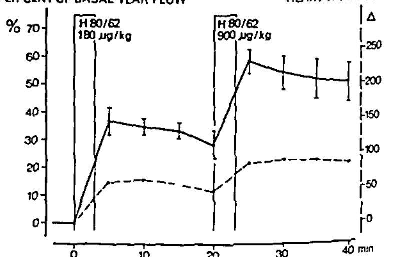


Fig 4

Effects of H 80 62 on basal tear flow (N = 6) and heart rate (N = 3) when given intravenously in the doses 180 + 900 mg/kg The sem values for heart rate were consistently less than 10 per cent of the mean values shown in the figure

flow was increased maximally by 58 ± 4 per cent (N = 6) The maximal ease in heart rate was 62 ± 4 beats/min after H 80/62 in the lower dose 3) and 92 ± 6 beats/min after the higher dose (N = 3) (Fig 4)

cts of metoprolol on H 80/62 induced increase in tear flow The H 80/62 ced increase of the tear flow was dose dependently decreased by meto ol 0 3–10 mg/kg The ED 0 value for metoprolol in these experiments was oximately 1 0 mg/kg (Figs 5 and 6)

xperiments with isolated rabbit lacrimal glands

spontaneous release of [³H]choline from isolated lacrimal glands was oximately 10 times higher than the release of [³H]acetylcholine Electrical l stimulation with 4 Hz 1 ms and with 10 Hz 1 ms induced a four fold ly nine fold increase in the release of [³H]acetylcholine (Figs 7 8)

oprenaline (3×10^{-8} mol/l) had no significant effect on the [³H]acetyl ine release neither during unstimulated conditions (Fig 7) nor during inuous stimulation at 4 Hz (Fig 8) The release of [³H]choline was how in both instances increased by isoprenaline

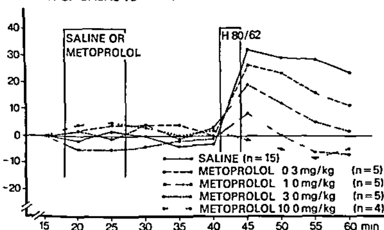


Fig 5

ects of metoprolol 0 3 1 0 3 0 and 10 0 mg/kg on H 80/62 induced increase of r flow After the infusions of H 80 62 the SEM values were consistently less than 5 per cent of the basal tear flow

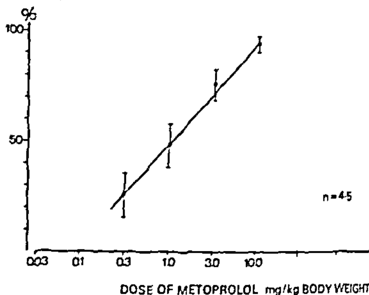


Fig 6

Inhibitory effect of metoprolol on H 50 62 induced increase of tear flow

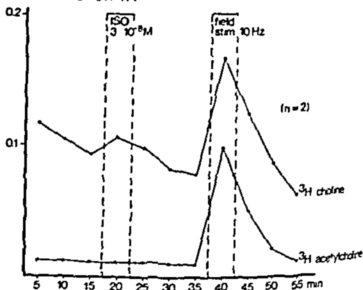


Fig 7

Release of [³H]acetylcholine and [³H]choline from isolated rabbit lacrimal gland. The preparations were stimulated first by isoprenaline (3×10^{-8} M) for 5 min and then electrically (10 Hz 1 0 msec) for 5 min as indicated in the figure.

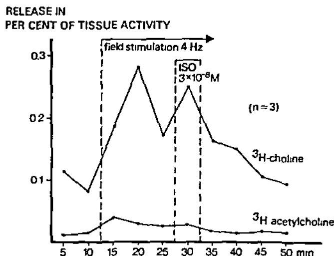


Fig 8

lease of [³H]acetylcholine and [³H]choline from isolated rabbit lacrimal glands ese preparations were given a continuous electric field stimulation (4 Hz 1 0 msec) d isoprenaline was added to the bath solution for 5 min as indicated in the figure

Discussion

ne main nervous supply to the lacrimal gland is of parasympathetic origin emtschenko 1872) The existence of a sympathetic nerve supply to the lacri al gland is however under discussion Thus from electrophysiological ex riments Botelho et al (1966) concluded that if sympathetic nerve impulses fluence the secretory activity of the lacrimal gland these impulses do not ach the gland via the lacrimal nerve After cannulation of the excretory ict of the inferior lacrimal gland an increase in tear flow has however been corded after parenteral administration of noradrenaline to anaesthetized rab ts (Goldstein et al 1967) In addition to these findings Botelho et al (1973) ve demonstrated that not only noradrenaline but also isoprenaline may in ease the tear flow in anaesthetized rabbits isoprenaline being even more fective than noradrenaline Botelho and her co workers also showed that the crease in tear flow produced by these drugs was abolished by the adrenergic blocking compound sotalol They concluded that in the rabbit lacrimal gland ere are β adrenergic receptors which are most likely located in the secretory lls The findings of Botelho et al (1973) have recently been verified by oissier et al (1976) They found that an isoprenaline induced increase in the ar flow in rabbits was decreased by propranolol

In the present investigation we found a dose dependent inhibition of isoprenaline induced increase in tear flow by propranolol as well as the selective antagonist metoprolol (Ablad et al 1973) The ED_{50} values for inhibition was 1 0 and 5 0 mg/kg respectively for propranolol and metoprolol (Fig 3) That relation between the two ED_{50} values is approximately the same as the relationship between their inhibitory activity against isoprenaline induced cardiac effects (Ablad et al 1973) This indicates that the adrenergic β receptors involved in lacrimal secretion were of the β_1 type In an additional series of experiments it was found that the tear flow was increased by the β_1 selective agonist H 80 62 (Carlsson et al 1977) and this increase was moreover blocked by the β_1 selective antagonist metoprolol We therefore conclude that the β receptors involved in the regulation of tear flow are of the β_1 type

The localization of these β_1 receptors is still unclear Theoretically they can be located either at the lacrimal secretory cells and directly influence the activities of the cells or they may be located at the cholinergic neurons and influence the release of acetylcholine In our *in vitro* experiments isoprenaline did not change the amount of acetylcholine released during electrical stimulation Therefore we are inclined to dismiss prejunctional sites on the cholinergic nerve endings as possible locations for the β_1 receptors although it was an interesting finding that [^{3}H]choline in these experiments was liberated when isoprenaline was added to the bath fluid Thus the most probable site for the tear flow regulating β_1 receptors in the intact animal are the lacrimal glandular cells There may also be vasodilating β_1 receptors in the lacrimal gland tissue as demonstrated for canine adipose tissue by Belfrage (19[illegible]

It may be concluded from the present investigation that the decrease in tear flow induced by adrenergic β blockers in this study was a pharmacological effect depending on an inhibition of β_1 receptors and not an unspecific toxic effect elicited by the β blocking agent

Acknowledgment

Thanks are due to Professor Erik Nilsson Linkoping and Dr Mogens Norn Copenhagen for stimulating discussions and friendly support

References

Ablad B Carlsson E Ek L (1973) Pharmacological studies of two new cardioselective adrenergic β receptor antagonists Life Sci 12 107–119

ırage E (19ı8) Comparıson of β adrenoreceptors mediatıng vasodılatatıon ın canıne ıbcutaneous adıpose tıssue and skeletal muscle *Acta physıol scand 102* 469–4ı6
ııer J R Advenıer Ch & Ho S (19ı6) Secretıon lacrymale chez le lapın et recep urs β adrenergıques *J Pharmacol (Parıs)* 941–250
elho S Y Goldsteın A M & Martınez E V (19 3) Norephrıne responsıve β drenergıc receptors ın rabbıt lacrımal gland *Amer J Physıol* 224 1119–1122
elho S Y Hısada M & Fuenmayor N (1966) Functıonal ınnervatıon of the lacrı ıal gland of the cat Orıgın of secretomotor fıbers ın the lacrımal nerve *Arch ıphthal (Chıcago) 76* 581–588
lsson E Dahlof C G Hedberg A Tångstrand B & Persson H (197ı) Dıfferen ıatıon of cardıac chronotropıc and ınotropıc effects of β adrenoreceptor agonısts *lrch Pharmacol 300* 101–105
ntschenko J (1872) Zur Innervatıon der Tranendruss *Pflugers Arch ges Physıol* 191–200
ldsteın A M de Palau A & Botelho S Y (1967) Inhıbıtıon and facılıtatıon of ılocarpıne ınduced lacrımal flow by norepınephrıne *Invest Ophthal 6* 498–511
rn M S (19ı4) *External eye Methods of examınatıon* Scrıptor Copenhagen ırmer O (1903) *Studıen zur Physıologıe und Pathologıe der Tranenabsonderung* und Tranenabfuhr *von Graefes Arch Ophthal 56* 19 –291
kberg J (1977) Release of [^{3}H]acetylcholıne from ısolated guınea pıg ıleum A adıochemıcal method for studyıng the release of the cholınergıc neurotransmıtter n the ıntestıne *Acta physıol scand 101* 302–317
kberg J E S (19 9) Synthesıs of [^{3}H]acetylcholıne ın the rabbıt lacrımal gland nd ıts release by electrıcal fıeld stımulatıon *Acta physıol scand* (ın press)
ıght P (1978) Untoward effects assocıated wıth practolol admınıstratıon Oculo nucocutaneous syndrome *Brıt Med J 1* 595–598

tho s present address
nnar Åberg Ph D
tra Pharmaceutıcal Products Inc
asant Street Connector
amıngham Mass 01701
S A

University Hospital Uppsala Sweden
Department of Ophthalmology (Head Lennart Berggren)

THE EFFECT OF METOPROLOL ON INTRA OCULAR PRESSURE IN GLAUCOMA

A Pilot Study

BY

ALBERT ALM CARL PETER WICKSTROM CURT EKSTRÖM and LENA ÖHMAN

Eleven patients with previously untreated glaucoma of one eye were given metoprolol 50 mg × 3 for one day Intraocular pressure (IOP) and heart rate were significantly reduced during treatment and increased after withdrawal of the drug In the eyes with glaucoma the mean IOP was reduced from 30 1 to 20 6 mmHg during treatment Systolic and diastolic blood pressures were also reduced during treatment but stayed at the same level after withdrawal of the drug It is concluded that at least part of the reductions observed for IOP and heart rate are drug induced while the effect on blood pressure is mainly induced by factors other than the drug The effect on IOP is considered large enough to be of clinical interest

Key words beta adrenergic blockers metoprolol glaucoma – intraocular pressure

It has previously been shown that beta adrenergic blockers reduce intraocular pressure (IOP) whether they selectively block β_1 receptors (Vale & Phillips 1973 Wettrell & Pandolfi 1977) or both β_1 and β (Phillips et al 196 Zimmerman & Kaufman 19//) Metoprolol (Seloken ®) is a selective β_1 antagonist without intrinsic activity and with only weak membrane stabilising properties (Ablad et al 1973) and the present study was undertaken to determine whether IOP is reduced by metoprolol The effect on heart rate and blood pressure was also determined

Received Ochtober 9 19 8

Material and Methods

ients were chosen according to the following criteria

lusion criteria 1) Previously untreated open angle glaucoma with or with lens exfoliations The diagnosis was based on the existence of glaucoma s visual field defects or uniocular IOP above 30 mmHg on repeated deter iations 2) IOP 30 mmHg or more measured at least on two occasions during first 24 h at the hospital IOP was measured every third hour except be en 12 p m and 6 a m

lusion criteria 1) Atrioventricular block seen on ECG 2) IOP more than mmHg 3) Arterial blood pressure above 180 mmHg systolic and/or 95 mmHg stolic 4) Previous treatment with beta adrenergic blockers 5) Diabetes mel s bronchial asthma or manifest cardiac incompensation

scription of the material A total of 11 patients consented to enter the study ey were 5 men and 6 women aged 51 to 82 years IOP was determined in h eyes with one exception (one eye was excluded due to chronic iridocyc s) The main object of the present study was to obtain information about effect on glaucomatous eyes Eleven eyes one from each patient suited inclusion criteria and were treated as one group in the statistical calcula ns This group will in the following be referred to as eye 1 For the re ining 10 eyes the highest IOP observed was 27 mmHg Statistical calcula ns for this group will be presented as eye 2

The study lasted three days not including the first day at the hospital which s used to determine the diurnal pressure curve the ECG and to inform the tient of the study

y 1 IOP was determined at 8 a m 2 p m and 8 p m with a Goldmann planation tonometer IOP was determined by one of the authors Heart rate d blood pressure were determined at 8 a m and 8 p m by a nurse and at p m by one of the authors Heart rate and blood pressure were determined ter at least 5 min rest

y 2 50 mg metoprolol (Seloken®) was given orally at 6 a m 12 and 6 p m P heart rate and blood pressure were determined as on day 1

y 3 No metoprolol was given and measurements were made as on days 1 id 2 in all patients except one where no measurements were made at 8 p m

During this time no other glaucoma therapy was given After the study the tients were questioned about side effects

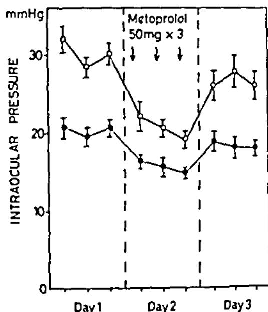


Fig 1

The IOP mean ± SEM in the glaucomatous eyes (eye 1 – open circle) and the f eyes (eye 2 – closed circle) at 8 a m 2 and 8 p m for three days Metoprolol was given 2 h before measurements of IOP on day 2 (arrows)

Results

Fig 1 shows the results of the IOP determinations at 8 a m 2 p m and 8 p for the three days of the study Mean values and SEM are given Fig 2 & the corresponding values for heart rate and blood pressures

The mean values observed at 8 a m 2 and 8 p m did not differ significan from each other on either day or for either parameter studied (Figs 1 and The mean pre treatment values calculated from the average of the th measurements on day 1 were IOP eye 1 30 1 ± 1 2 mmHg eye 2 20 3 ± 1 mmHg systolic blood pressure 154 ± 3 mmHg diastolic blood pressure 87 2 mmHg and heart rate 74 ± 2 (Mean ± SEM) Averaged values for day 1 and 3 were used to calculate the differences observed between the various d Table I presents such differences based on paired data The difference (d 1 day 2) shows the change observed when the drug was given and the differen (day 3 – day 2) the degree of recovery when the drug was withdrawn IOP both eyes and heart rate were reduced on day 2 and showed an incomple recovery during day 3 All differences were statistically significant Sys

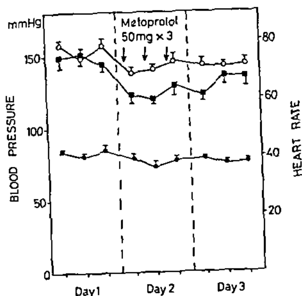


Fig 9

[He]art rate and blood pressures Times for measurement and administration of metroprolol as in Fig 1 Mean and 1 SEM are presented

- Systolic blood pressure
- Diastolic blood pressure
- Heart rate

Table I

[Di]fferences based on paired data for the mean values of day 1 2 and 3 respectively [M]ean ± SEM (n = number of patients) and statistical significance (Student s *t* test) n. s not significant

[D]ays for which the difference is calculated	IOP Eye 1 mmHg (n = 11)	IOP Eye 2 mmHg (n = 10)	Heart rate (n = 11)	Syst BP mmHg (n = 11)	Diast BP mmHg (n = 11)
Day 1 – Day 2	9.5 ± 1 1 *P* < 0 001	4 7 ± 0 9 *P* < 0 001	12 0 ± 1.3 *P* < 0 001	12 3 ± 3 4 *P* < 0 01	6.5 ± 1 8 *P* < 0 005
Day 3 – Day 2	6 3 ± 1 1 *P* < 0 001	2 7 ± 0 6 *P* < 0 005	3 6 ± 1 3 *P* < 0 025	1 2 ± 2 2 n s	0 2 ± 2 2 n s

and diastolic blood pressures also showed a significant reduction on day 2 but no recovery was observed on day 3

One patient complained of slight fatigue during day 2 but otherwise no side effects were observed

Discussion

The aim of this pilot study was to examine the possibility that metoprolol has an IOP lowering effect large enough to be of clinical interest The dose 150 mg is comparable to 160 mg propranolol regarding the effect on heart and blood pressure (Johnsson et al 1975) and it was chosen since 80 mg propranolol twice daily is known to have a marked possibly maximal effect on IOP (Wettrell & Pandolfi 1976, Borthne 1976) IOP was determined 9 h after administration of the drug At this time the effect of the drug can be expected to be almost maximal

In the present study significant reductions were observed for all parameters observed on day 2 but the degree of recovery on day 3 varied (Table I) A significant recovery on day 3 indicated that at least part of the observed effect on day 2 is drug induced while no recovery may be explained by the possibility that the effect observed on day 2 is a placebo effect or that the drug effect still remains Since the time for a 50 % reduction of the maximum effect of 100 mg metoprolol on exercise heart rate and blood pressure is about 8 h (Regårdh et al 1975) it is possible that some drug effect remains at 8 a m on day 3 but not likely at 8 p m Table I presents only the differences based on the mean values for the different days but as seen in Figs 1 and 2 no significant difference in recovery was observed between 8 a m and 8 p m on day 3 with the possible exception of heart rate Thus it is possible that for heart rate part of the incomplete recovery on day 3 is due to remaining drug effect at 8 a m resulting in a disproportionate low mean value In fact the difference in heart rate between day 1 and 3 at 8 p m is not significant (4 6 ± 2 4 0 05 < P < 0 1 Student s t test paired data) It may then be concluded that in the present study metoprolol reduced IOP and heart rate while the blood pressure reduction observed on day 2 and 3 was mainly induced by factors other than the drug e g decreased anxiety in these patients with a recently diagnosed glaucoma The conclusion that the observed blood pressure reduction was not drug induced may be surprising considering the known blood pressure reducing effects of various beta adrenergic blockers including metoprolol (Berglund 1976) However in the present study blood pressure was determined at rest in patients without known arterial hypertension and after only one days d

ı nıstratıon The exact mechanısm by whıch beta adrenergıc blockers reduce ›d pressure ıs not known but ıt ıs belıeved to be secondary to a reductıon ardıac output and renın release (Sımpson 19/4) and ın persons wıth normal ›d pressure ıt ıs most marked durıng exercıse and may have no hypotensıve ct ın rest (Schroder & Werko 1964) An ınıtıal blood pressure reductıon / not be observed even ın patıents wıth arterıal hypertensıon sınce the re ed cardıac output ıs at fırst compensated by an ıncreased perıpheral resıs e (Hansson et al 1974) whıch wıll gradually return towards the ınıtıal ıe or even less Stıll there ıs no reason to doubt that long term oral treat ıt of metoprolol ın patıents wıth glaucoma wıll cause a true reductıon of ›d pressure and wıth metoprolol as wıth other beta adrenergıc blockers ıcal applıcatıon of eye drops would be preferable sınce a reduced blood ssure may dımınısh the benefıt of a reduced IOP

The effect on IOP ın the present study ıs consıdered large enough to be of ıcal ınterest and ıt ıs of the same order as found for propranolol ın a sımılar ly (Borthne 19/6) However untıl dose response relatıonshıp and duratıon e been determıned meanıngful comparısons cannot be made wıth other beta energıc blockers Sınce the completıon of the present study Ros et al (19/8) e reportec the effect of metoprolol eye drops These ınvestıgators found a xımum effect for 1 per cent metoprolol wıth a duratıon of at least 8 h but sımılar study on oral admınıstratıon of metoprolol ıs known to us The re :s of the present study however encourage further studıes on the effect of toprolol on normal and ıncreased IOP

Acknowledgment

: authors wısh to thank AB Hassle Molndal Sweden for the supply of metoprolol lets

References

lad B Carlsson B & Ek L. (1973) *Pharmacologıcal studıes of two new cardıo-selec ıve adrenergıc beta receptor antagonısts* *Lıfe Scı 1° part I* 10/–119

ıgtsson C. (19/6) The effect of metoprolol – a new selectıve adrenergıc beta₁ recep or blockıng agent – ın mıld hypertensıon *Acta med scand 199* 6>–/0

rthne A (19 6) The treatment of glaucoma wıth propranolol (Inderal®) A clınıcal ınal *4 ta ophthal (Kbh) 54* °91–300

ınsson L. Zweıfler A J Julıus S & Hunyor S N (19 4) Hemodynamıc effects of ıcute and prolonged β adrenergıc blockade ın essentıal hypertensıon. *Acta med scand 196* 2/–34

Johnsson C Nyberg C & Solvell I (1975) Influence of metoprolol and pr on hemodynamic effects induced by physical work and isoprenaline *Acta pharmacol et toxicol 36 Suppl V 0-75*
Phillips C I Howitt G & Rowlands D J (1967) Propranolol as ocular hyp agent *Brit J Ophthal 51 222-226*
Regårdh C G Johnsson C Jordo L & Solvell I (1975) Comparative bioavail and effect studies on metoprolol administered as ordinary and slow release tablets single and multiple doses *Acta pharmacol et toxicol 36 Suppl V 45-58*
Ros F E Dake C L Nagelkerke N J D & Greve E L (1978) Metoprolol e in the treatment of glaucoma A double blind single dose trial of a beta adrenergic blocking drug *Albrecht v Graefes Arch klin exp Ophthal 206 241-254*
Schroder G & Werko L (1964) Nethalide a beta adrenergic blocking agent *Pharmacol and Ther 5 159-166*
Simpson F O (1974) β adrenergic receptor blocking drugs in hypertension *Dr 85-10*
Vale J & Phillips C I (1973) Practolol (Eraldin) eye drops as an ocular hypotensive agent *Brit J Ophthal 57 210 214*
Wettrell K & Pandolfi M (1976) Early dose response analysis of ocular hypotensive effects of propranolol in patients with ocular hypertension *Brit J Ophthal 60 680 683*
Wettrell K & Pandolfi M (1977) Effect of topical atenolol on intraocular pressure *Brit J Ophthal 61 334-338*
Zimmerman T J & Kaufman H E (1977) Timolol A beta adrenergic blocking agent for the treatment of glaucoma *Arch Ophthal (Chicago) 95 601-604*

Authors address
Albert Alm M D Carl Peter Wickstrom M D
Curt Ekstrom M D and Lena Ohman M D
Department of Ophthalmology
University Hospital
S 750 14 Uppsala 14
Sweden

The Department of Ophthalmology
(Heads V Dreyer J Edmund E Gregersen
S V Kessing H H Seedorff)
Rigshospitalet University of Copenhagen

CORNEAL ASTIGMATISM AFTER CATARACT EXTRACTION

A Comparison of Corneal and Corneoscleral Incisions

BY

JOHN THYGESEN PER REERSTED HANS FLEDELIUS
and LEIF CORYDON

Corneal astigmatism after cataract surgery by way of corneal incision (C n = 62) and corneo scleral incision (CS n = 61) was followed for six months Corneal incisions were closed by continuous nylon 10–0 (–25 loops median 14) Corneo scleral incisions were sutured with single knots (2–10 median value 5)
Keratometric results in the C and CS groups are compared Concerning the *degree* of astigmatism pre operative median values were 0.5 and 0 7 D respectively After one week they were 4 5 and 3 0 D after two weeks 3.3 and 3 0 D after four months 3 0 and 2 0 D after six months (final status) 1.5 and 1 D respectively The differences between C and CS were not significant For both astigmatism after cataract surgery did *not* quite return to pre operative levels
Concerning the *axis* (weaker meridian) of corneal astigmatism the C cases retained their pre operative distribution while the CS cases showed the classical shift towards *against rule* astigmatism
Final corrected visual acuity was of the same order in the C and CS group
Due to frequent shifts also of the axis it is recommended by early (pre liminary) glass prescription *not* to correct the astigmatism but to give only the best spherical correction

Key words cataract extraction – corneal incision – corneo scleral incision – corneal astigmatism

he material was presented to the Danish Ophthalmological Society April 15 19/8

eceived September 22 19/8

During the last years a corneal incision (under the microscope) has found creasing use in our department in cataract surgery Inevitably discussion concerning the advantages and drawbacks as compared with the routine thod in use until then namely an ab externo corneo scleral incision a limbus based conjunctival flap It thus appeared that corneal astigmat often high after a corneal incision at least initially and apparently the p had to wait longer for definitive glass prescription

To clarify these points a consecutive series of cataract extraction by the methods was followed for six months and the results were compared T degree of and the change in post operative corneal astigmatism is the subj of the present publication

Materials and Methods

123 consecutive cataract extractions from May to November 1976 were included the study

Excluded were only 1) cases in which keratometry was not possible due to existing corneal pathology and 2) patients where geography was inhibitory to fo up (Greenland Faroe Islands remote parts of Denmark)

The bulk of the material was made up of senile and presenile cataracts but there were also some complicated cataracts (uveitis excessive myopia heterochromia) Ca with complications during surgery were not excluded

Age of patients ranged from 33-91 years in the corneal and 54-87 years in the corneo scleral group There were 63 female and 60 male eyes 67 right and 56 l eyes

62 patients had a corneal incision and a continuous nylon 10-0 suture except six cases with single sutures (virgin silk vicryl dexon) The number of loops 7-23 median value 14

61 patients had a corneo scleral incision under a limbus based conjunctival fl Single sutures were used in a number from 2 to 10 median value 5 Virgin silk used in 33 vicryl in 22 and collagen in 6

The incision was made ab externo in two steps and angled first with knife (a razer blade perpendicular to surface to a depth of ²/₃ of the cornea) and then scissors All cases but six were operated on under the microscope

The operations were performed – usually in local anaesthesia – by members of the staff a) Chief surgeons 57 cases (38 corneal 19 corneo scleral) and b) Resi 66 cases (24 corneal 42 corneo scleral)

There was a slight load of heavy cases in the corneal group (because in gene a corneal incision was chosen in cases where complications were expected) According ly vitreous loss occurred more often in this group (7/62) than in the corneo-scle group (2/61) a transitory rise in IOP (above 24 mmHg) was found in four and one eye respectively There was one case of flat chamber after surgery (corneo-scleral incision) and one case of secondary haemorrhage (corneal incision) Clip lenses w implanted in seven corneal and in two corneo scleral cases

amination scheme

< (corneal astigmatism in dioptres) the axis (weaker corneal meridian in degrees) d corneal power (average between weaker and stronger meridian in dioptres) were asured by Javal Schiøtz *keratometry* 1) pre operatively 2) one week after 3) two eks after 4) four months after (where the nylon suture was removed in corneal ses) and 5) six months after surgery (defined as final status)

On the last occasion *refraction* (spherical equivalent in dioptres) and corrected *sual acuity* were also recorded

atistical methods

ata were put on punch cards Standard computer programs disclosed that *non para tric* statistics were to be preferred (due to the distribution of the two main para ters the keratometric ΔK value and the orientation of the axis) P values are given ien significant ($P < 0\ 05$)

Results

t the post operative follow up examinations there were a few fall outs a ct that is reflected in the varying numbers of eyes shown in Fig 3

In the following the designations C and CS will be used for the corneal id corneo scleral group respectively

Table I shows the corneal astigmatism before surgery and on the four post ›erative occasions Mean values and standard deviations are shown to allow ›mparison with earlier relevant studies but the median value is preferred as more true indicator Prior to surgery there is no difference between the C

		Pre op	1 week	2 weeks	4 months	6 months
ΔK in Dioptres MEAN VAL ± SD	Corneal	10 ±10	42 ± 23	40 ± 24	37 ±24	22 ±17
	Corneo-scleral	09 ± 08	40 ± 29	36 ± 32	23 ±16	20 ±16
ΔK in Dioptres MEDIAN VAL (range)	Corneal	05 (0 45)	45 (05 10)	33 (05 9)	30 (0 11)	15 (0 65)
	Corneo scleral	07 (0 40)	30 (0 12)	30 (0 18)	20 (05 7)	17 (0 80)

Table I

orneal astigmatism ΔK before and after cataract extraction on four post operative ccasions Mean values and SD (top) median values and range (bottom) Corneal and corneo scleral cases

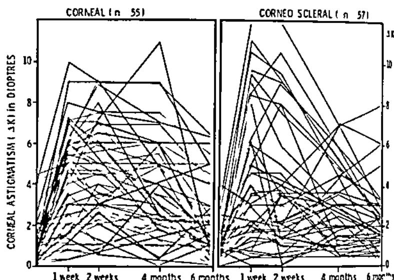


Fig 1
Corneal astigmatism before and after cataract extraction recordings of single cases Corneal group left corneo scleral group right

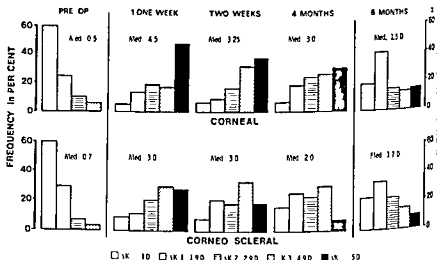


Fig 2
Corneal astigmatism prior to surgery (left) and on four occasions after cataract extraction Percentage distribution on five ΔK classes Corneal cases (top) corneo-scleral cases (bottom)

l CS group and the same applies to the final ΔK value The four months tus however leaves the impression that CS cases achieve their final ΔK ue somewhat earlier than the C cases

The same is evident from Figs 1 and 2 which show longitudinal recordings single cases (Fig 1) and percentage distributions (Fig 2) In Fig 1 there in the C group a rather high ΔK plateau until suture removal (after four nths) while normalization occurs earlier among those with CS According the darker (less favourable high ΔK classes) columns of Fig 2 show an rupt increase in relation to surgery followed by a gradual return to a dis oution which comes closer to the status before surgery

Assessed by χ tests there is no significant difference between C and CS on five occasions i e by vertical comparisons of the distributions shown in 2 By hori ontal assessment – *within* the C and CS group respectively – re is a highly significant difference between the ΔK distribution before o and at the final status ($P < 0\ 001$) and this applies to both groups rneal astigmatism thus does not return to preoperative levels

The axial orientation of corneal astigmatism (position of weaker meridian) s recorded on the keratometer and transferred to punch card in its relevant degree box (see Fig 3) Astigmatism with the rule comprised cases th the axis between 0–20° and 160–180° Against the rule broadly com

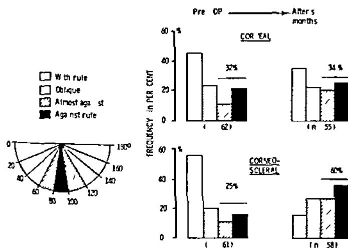


Fig 3

Axial orientation of corneal astigmatism prior to surgery for cataract (left) and 6 months after surgery (right) with corneal cases (top) and corneo scleral cases (bottom) Percentage distributions

prised the zone 60–120° but against" in a narrow sense (90 ± 10) is separated and specified with black in Fig 3 Oblique astigmatism occupies the remaining 2 x 40°

Before surgery about 50 % were with rule and there was no difference between C and CS groups At the final status after six months the change of axial orientation (distributions of Fig 3) was not significant within the C group which maintained a ¹/₃ share of "against rule" astigmatism Conversely there was a significant shift ($P < 0.001$) in the CS group with a marked reduction in with rule" astigmatism and a marked rise in the against rule fraction (from 25 to 60 %) The final C and CS status differ accordingly but at only a 0 05 level

The material enables us to give an answer to a practical question Can glasses be prescribed early? Because if significant changes occur in only a *minor* fraction the major part of patients might have the benefit of an early full prescription This however did not hold for any of the groups when *single* cases were followed (in contrast to the summary distributions of Fig) As concerns IK about 50 % had changes exceeding 2 dioptres between day 14 after surgery and six months Further about fifty per cent showed an axis shift exceeding 20 degrees in the same period

The above results are considered the main object of the present study In addition a summary mention will be given of some other data

Corneal power (range 39 3–48 5 D) did not change much in relation to surgery After exclusion of one eye with a presumed faulty recording (of 35 5 in the C group) the C cases had the mean value 43 28 D (SD 1 66) before surgery and 43 13 (SD 1 99) after six months The corresponding CS values were 43 06 (SD 1 66) and 43 09 D (SD 1 89)

Final corrected *visual acuity* was good in both groups with values ≥ 6/9 84 % Visual acuity ≤ 6/60 (pre existing maculopathy 2 cases aphakic retinal detachment 1 case) occurred in one eye with C and in two with CS

There was no correlation between astigmatic error (IK) and final corrected visual acuity

After exclusion of the nine eyes with clip lenses the *final refraction* (spherical equivalents) of C cases showed a significant shift ($P < 0.01$) towards lower values when compared with the CS group This is reflected also in the mean values of the two groups being 11 1 D (SD 3 3) and 12 9 D (SD 1 5) respectively As deducted from the aphakic refractive values the C group thus entailed eight eyes that had high myopia prior to surgery against only one eye in the CS group

The final post operative astigmatism showed no correlation (Spearman rank) to pre operative corneal astigmatism age of patient number of suture loops or to visual acuity (vide supra)

Discussion

dvances in surgery (microsurgical techniques) and pharmacology (access to itibiotics and corticosteroids) have by and large eliminated the formerly eaded severe complications of cataract extraction Therefore with the words Troutman (1970) we can now begin to worry about the previously neglec d details One area which has been ignored is the optical distorsion of the rnea randomly induced by current cataract incision and closure over which ance still has the best control

This degree of corneal distortion is the sole subject of the present publica on which does *not* deal with other aspects of cataract surgery Let it only stated that a corneal incision has been used because in general this is re irded as being pretty safe (Corydon 1976 Corydon & Mackensen 1978) and pecially so in eyes with expected complications to surgery (a fact that is also flected by the greater share of heavy cases in the corneal group of the present aterial)

The ideal set up for a comparison of the two cataract surgery incisions would as follows The same surgeon should perform the surgery in all cases and ith equal skill and experience as regards the two incisions to be compared llocation to the two techniques should be strictly at random Regard should so be taken to type of knife and scissors to suture material and technique well as to the age of the patient sex type of cataract eye size etc

Such demands are hard to fulfil in a clinical series This is apparent from e present material as well as those of earlier reports on the subject We anaged to keep the unity of time and place but a considerable number of e surgeons were included experienced as well as residents and the tech ques of wound closure varied Corneal incisions were usually closed by a ntinuous nylon suture while corneo scleral incisions were sutured by single itures in a varying number and with different materials

The present approach is therefore primarily practical clinical and does not iminate multifactorial hazards but we still consider a comparison of the two ethods as being justified This may be supported i a by the fact that the rneal astigmatism after surgery by residents equalled the results gained by ie chief surgeons A similar experience was reported earlier by Bedrossian al (1969)

From our results we may advance that the *final* degree of corneal astigma sm showed no relation to the type of incision Further our 6 months values qualled those of a quite recent German study (Steinbach & Gerhard 1978) ith a set up quite similar to the present one In both studies however nor

malization seemed somewhat retarded in the group with corneal incision due to the late removal of the non absorbable nylon suture

Concerning the axis of astigmatism there is however a remarkable difference between the two studies Steinbach & Gerhardt (1978) found – *for both incisions – a shift in axis from with the rule towards against rule (weakest meridian vertically)* This is the classical experience gained since the Graefe incision was introduced but it does not accord with more recent reports that emphasise a more natural tendency" (with rule) after corneal incision with meticulous wound closure under the microscope In our material this was not evident We found a significant difference between the classical against rule corneo scleral cases and those with a corneal incision The latter largely kept *their original distribution of axial directions (Fig 3)*

The impact of the present study is that the end results concerning corneal astigmatism after the two different incisions were pretty similar however with the exception mentioned regarding the axis This had but little influence on the functional end result (which is corrected visual acuity) We think like Steinbach & Gerhardt (1978) – that both methods are from an astigmatic point of view equally suited for cataract surgery And further modern microsurgical *advances have now modified one of the classical dogmas* about cataract surgery that astigmatism will increase the more corneal (nearer to vertex) the incision is located (Lundsgaard 1925)

It is tempting finally to quote another countryman Edmund who at the same meeting in 1925 stated that "Nature has a peculiar ability to let a by external actions altered anatomy return to its original norm

References

Bedrossian P B Sabater G & Troutman R C (1969) *Induced astigmatism as a result of surgery* South African Intern Ophthal Sympos 219 25 Butterworth Washington

Corydon L (1976) *Komplikationen bei Kataraktoperationen* Inaug Diss Freiburg

Corydon L & Mackensen G (1978) Progress in cataract surgery using microsurgical technique *Acta Ophthal (Kbh)* 5 53–66

Edmund C (1925) *Det oftalmologiske Selskabs forhandlinger 1924–1925 (Kbh)* 3

Lundsgaard K K K (192) Den postoperative Astigmatisme *Det oftalmologiske Selskabs forhandlinger 1924–25 (Kbh)* 16–28

Steinbach P D & Gerhardt G (1978) Postoperativer Astigmatismus und Visus nach Kataraktextraktion bei unterschiedlichem operativem Vorgehen *Klin Mbl Augenheilk* 172 305–312

Troutman R C (1970) Control of corneal astigmatism in cataract and corneal surgery *Trans Pacific Coast Oto Ophthal Soc* 51 217–231

• addition, reference is made to essential contributions in a recent publication *rrent Concepts in Cataract Surgery* (Emery J M and Paton D Eds 1976 C V ›sby) which came to our knowledge after the present paper was finished

thors addresses

hn Thygesen,
e Dept Hvidovre Hospital
penhagen
ttegård Alle
2650 Hvidovre
nmark

r Reersted,
e Dept Gentofte Hospital
(2900 Hellerup
nmark

The Department of Ophthalmology (Head N Ehlers)
Århus Kommunehospital University of Aarhus Denmark

EARLY POSTOPERATIVE CHANGES IN GRAFT THICKNESS AFTER PENETRATING KERATOPLASTY

Influence of Host Corneal Disorder on Time Course

BY

THORKILD BRAMSEN and NIELS EHLERS

In 1 2 patients the thickness of the corneal graft was followed with fre quent measurements during the first 14 days after operation. Three dif ferent graft thickness time courses were observed Patients with keratitis stromal dystrophy and corrosion or mechanical lesion showed a secondary rise in graft thickness on the 6th postoperative day while patients with keratoconus and those treated with tranexamic acid showed no rise on the 6th day Patients with Fuchs dystrophy differed from the other groups in not reaching the maximal thickness until the 3rd postoperative day

The possible correlation of these three time courses with changes in the fibrinolytic system is discussed

Key words transplantation – graft thickness – pachometry – fibrinolysis – keratoconus – Fuchs dystrophy – keratitis

The regulation of the corneal thickness after an operation or after an ocul inflammation is probably influenced by the same mechanisms which maint the normal thickness It may therefore be of interest to follow the time co of this normalization by frequent measurements of the central corneal thickn (CCT) after ocular surgery The most marked changes in CCT occur s after the operation and often the thickness is normal within a few we Changes in thickness may reflect a changing activity of the mechanisms co

Received May 31 1978

lling the thickness Various time courses of CCT after surgery in cases of ferent ocular disorders may give a clue to the pathogenesis of these con ions

nvestigations concerning the influence of the antifibrinolytic drug tran imic acid (Cyclokapron®) on the CCT after intraocular operations (Bram et al 1978 Bramsen 1978) and a study of the effect of this drug on CCT cases of Fuchs endothelial dystrophy (Bramsen & Ehlers 19//) have shown t tranexamic acid has a thickness reducing effect in pathological oedema l effect on the normal cornea has been examined in a double blind study (to published) revealing a quantitatively smaller but definite thickness reducing ect of tranexamic acid

These investigations due to the antifibrinolytic effect of tranexamic acid d support to the hypothesis that in man the fibrinolytic system is involved the regulation of the normal as well as the pathological corneal thickness ring the last five years we have regularly followed graft thickness in the t 14 days after corneal transplantation We report here an analysis of the irse of graft thickness during this time period in various groups of corneal t diseases and in a group of patients treated with tranexamic acid

Material and Methods

e material comprises 17_ patients subjected to penetrating corneal transplantation the period January 1st 19 3 to April 1st 19 8 Thirty of these patients were in ded in an earlier study of graft thickness (Ehlers 19 4) The technique of opera n and of measuring the graft thickness has been reported earlier (Ehlers 1974 lers & Sperling 197/) Almost all the measurements were made by the authors e graft thickness was measured several times during the 14 days of hospital stay er transplantation, and in a substantial number of cases it was measured almost ry day

The material has been divided into 6 groups 1) 66 cases of keratitis (herpetic and n herpetic) 9) 38 cases of keratoconus 3) 23 cases of Fuchs endothelial dystrophy 17 cases of stromal dystrophy 5) 16 cases of corrosion or mechanical lesion, and ally 6) 1 cases treated in the postoperative period by systemic tranexamic acid ly amount 5 mg/kg body weight divided in three doses) This latter group com ses 9 cases of keratitis 2 cases of Fuchs dystrophy 4 cases of stromal dystrophy d 9 cases of mechanical lesion Tranexamic acid treatment was started before the rd postoperative day often in cases with a fairly thick graft, as a therapeutic empt. This explains the rather high values of graft thickness on the first post erative day in this group For each of the 6 groups average donor age and graft e were computed Table I shows these data from which it may be seen that the oups are reasonably *comparable Analysis of variance revealed no significant* fference between the groups in respect to donor age or graft age

Table 1

An analysis of variance showed no heterogeneity among the age groups (F = 0.67 for the donor age groups, F = 0.95 for the graft age groups f_1 5 [illegible]

Diagnostic group	No	Donor age (years) $\bar{x}$ ± SEM	Graft age [illegible] $\bar{x}$ ± SEM
Keratitis	66	44.93 ± 2.13	[illegible]3.91 ± [illegible]
Keratoconus	38	39.38 ± 3.12	31[illegible] ± [illegible]
Fuchs dystrophy	23	40.55 ± [illegible].90	39[illegible] ± [illegible]
Stromal dystrophy	12	40.83 ± 5.5[illegible]	3[illegible]00 ± [illegible]
Corrosion or mechanical lesion	16	45.[illegible]4 ± 5.[illegible]9	346[illegible] ± [illegible]
Tranexamic acid treated	17	44.59 ± 4.2[illegible]	[illegible]3[illegible]94 ± [illegible]

Results

For each of the 172 patients the time course of the graft thickness was dr[illegible] Analysis of the curves in the 6 groups mentioned revealed three different [re]sponses. Fig. 1 illustrates the characteristics. Fig. 1 A is an example of graft thickness time course in a patient with keratitis. The steady fall is in[ter]rupted by a secondary rise beginning on the 5th postoperative day. After [the] 7th day a steady fall is again observed. Fig. 1 B shows the time course af[ter] grafting a patient with keratoconus. There is a steady fall during the obser[vation] 14 days and no secondary thickness increase around the 5th–6th day. Fi[g. 1 C] shows the time course after grafting a patient with Fuchs endothelial d[ys]trophy. The most remarkable in this curve is that the maximum thickness [is] found 2–3 days after the operation and not as in the other groups on the fi[rst] postoperative day. Fig. 1 D shows the time course after grafting a patient w[ith] keratitis treated in the postoperative period with tranexamic acid. This cur[ve] shows a course similar to that observed in patients with keratoconus.

The four examples are considered to be characteristic. In order to docume[nt] this statement, average curves have been computed for the 6 groups mention[ed] (Figs. 2–7). Within each group the average of all available measurements a[t] the days were calculated ($\bar{x}$ ± SEM). In Figs. 5 and 6 the number of measure[-] ments on the last days were limited and therefore the data were added f[or] several days and shown as value of the 11th day.

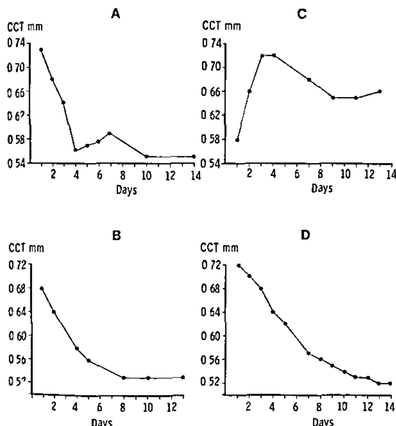


Fig 1

Graft thickness after penetrating keratoplasty Four examples of characteristic time course

A Corneal host disease keratitis

B Corneal host disease keratoconus

C Corneal host disease Fuchs endothelial dyhtrophy

D Tranexamic acid treated keratitis

The 6 average curves show the three characteristic time courses The groups f keratitis stromal dystrophy and corrosion or mechanical injury (Figs 2 5 nd 6) all show a secondary thickness increase at the 6th day The groups of eratoconus and tranexamic acid treatment (Figs 3 and 7) showed no secon ary rise in thickness at the 6th day The group of Fuchs endothelial dystrophy Fig 4) did not show the maximum thickness until the 3rd postoperative day

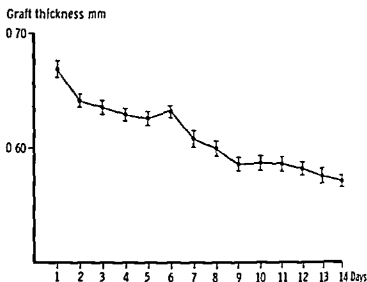


Fig 2
The average curve in the group of keratitis (x ± SEM)

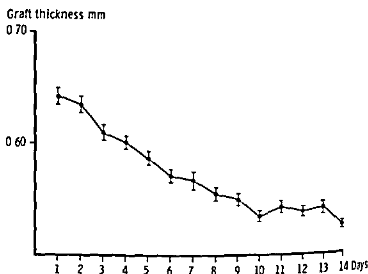


Fig 3
The average curve in the group of keratoconus (x ± SEM)

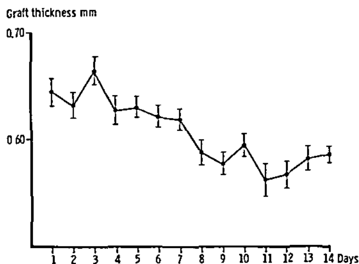


Fig 4
The average curve in the group of Fuchs endothelial dystrophy (x ± SEM)

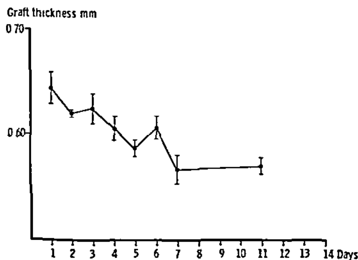


Fig 5
The average curve in the group of stromal dystrophy ($\bar{x}$ ± SEM)

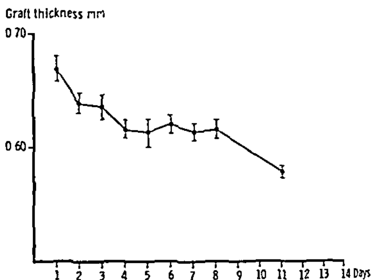


Fig 6
The average curve in the group of corrosion or mechanical lesion ($x \pm$ SEM)

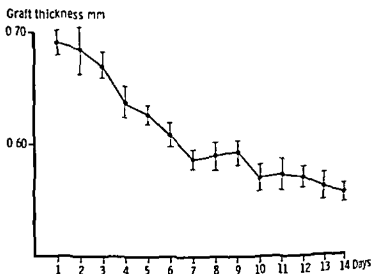


Fig 7
The average curve in the tranexamic acid treated group ($x \pm$ SEM)

Table II
Time course of postoperative graft thickness decrease

Diagnosis	No of cases	Steady fall	Secondary increase 4th to 7th day
Keratitis	66	1/	49
Keratoconus	38	30	8
Fuchs dystrophy	93	10	13
Stromal dystrophy	12	6	6
Mechanical lesion or corrosion	16	2	14
Tranexamic acid treatment	17	14	3
Total	1/2	/9	93

43 5 f = 5 $P \ll 0\,001$

rrom the 1/2 curves it was counted how many showed a steady fall and w many showed a secondary rise within the 4th to 7th postoperative day it is showed the time courses of Fig 1 A and B respectively The result pears in Table II Of the 66 cases of keratitis 49 showed a secondary rise ile in the group of 38 cases of keratoconus only 8 showed a secondary rise In tranexamic acid treated group only 3 of 17 showed a secondary rise Eva ation of the figures by χ test showed that the observed frequencies differed nificant from a random universical distribution ($P < 0\,001$)

Discussion

a previously published study (Ehlers 1974) the graft thickness was followed /0 patients 32 of these with regular measurements The purposes at that ne was to show the changes in graft thickness in the total series of patients d not any differences between various groups It was noted however that o types of postoperative graft thickness course occurred one showing a steady ll another with a secondary rise after about one week There were an ap oximately equal number of cases in the two groups

The present study has confirmed the existence of these two different p- operative thickness time courses Patients with keratitis with stromal dys- or with corrosion or mechanical lesion show a secondary transient rise 4 to 7 days after the transplantation while patients with keratoconus show such secondary rise

As a new observation it has been noted that patients with Fuchs endoth. dystrophy show a different course characterized by an increase in graft th. ness during the first postoperative days When a secondary rise occurs in t group it tends to be some days later than in keratitis Patients treated with antifibrinolytic drug tranexamic acid show a course almost identical to t. of keratoconus patients It may be emphasized that the tranexamic acid trea group contains no cases of keratoconus

The six groups did not differ with regard to donor age or graft age, a. is therefore tempting to seek an explanation of the different time course different responses to surgical trauma in the groups

Ygge (1970) followed the plasma content of fibrinolytic inhibitors after a dominal surgery He found that the antifibrinolytic activity was incre shortly after the surgical trauma and reached a maximum value after 3-4 d In the next days the inhibitory effect fell and was minimal after about 1 w The same time course was observed in patients subjected to injuries (Ram. & Saldeen 1970) Ygge (1970) also investigated the spontaneous fibri activity in serum and found a fall the day after the surgical trauma follow by an increase after 3-4 days occurring simultaneously with the fall in hibitors

These changes in fibrinolytic factors are understandable when the mechan of wound healing is considered A wound is primarily closed by fibrin, wh at first forms the only stabilization of the wound When vessels grow into t fibrin layer in 4-5 days the fibrin must be removed to allow the formation collagen It can therefore be considered appropriate to have a low fibrinoly activity in the first days after a lesion and a high activity after 5 days wh fibrin should be removed

Reme & Witmer (1974) showed that fibrin is formed in iris wound be- to disappear on the 5th day and is completely removed 7 days after th le-

The fibrinolytic system is therefore also active in the eye and the respo with stabilization and later break down of fibrin would seem to the same elsewhere in the body Studies on traumatic hyphaema (Bramsen 1976 19 have shown that the secondary haemorrhages are prevented by administe the antifibrinolytic drug tranexamic acid

It would seem therefore that an increase in fibrinolysis locally in the e as well as generally in the body occurs about 3-6 days after a lesion This

ased fibrinolytic activity occurs simultaneously with or shortly before an rease in corneal thickness

A common response in postoperative graft thickness is observed in patients h keratitis stromal dystrophy and corrosions and mechanical lesion The neal thickness is maximal the day after the operation and falls steadily until 5th day when a secondary and temporary increase occurs This increase is er a few days followed by a continuation of the steady fall This response rees with the variations observed in fibrinolytic activity in plasma after other uma The prevention of the secondary increase in thickness by antifibrino ic therapy supports this explanation The postoperative graft thickness course th a transient secondary increase 6 days after operation may be considered normal response This response can be altered by antifibrinolytic treatment

The graft thickness course in keratoconus patients is identical with that ob ved after tranexamic acid treatment The secondary rise in thickness is sent or possibly delayed It is well known that corneal grafting in kerato nus gives very favourable results We have noted that the graft in kerato nus eyes often becomes very thin The thickness time course in this group was derstandable if the patients with keratoconus had a low level of fibrinolytic tivity or an abnormal weak fibrinolytic response to injuries

The graft thickness time course in patients with Fuchs endothelial dystrophy ffers from all the other groups in the sense that the maximal graft thickness not gained until three days after the operation and not until after seven ys does the thickness show a fall In a previous study on Fuchs dystrophy ramsen & Ehlers 1977) it was shown that the corneal oedema could be re ced by means of antifibrinolytic treatment The graft thickness time course uld be understood if these patients opposite to keratoconus patients had a gh level of fibrinolytic activity or an abnormally large or prolonged fibrino tic response to injuries We are at present studying the variations in anti brinolytic factors in serum after corneal grafting (Bramsen & Stenbjerg 1979) hese patients have been selected because they routinely stay in hospital for o weeks and thus give the opportunity to follow changes by daily blood mples There is nothing however to suggest that the secondary rise in thick ss is confined to transplantations Mishima (1968) included a curve of corneal ickness after cataract extraction clearly showing a transient increase about ne week after operation

References

Bramsen T (19 6) Traumatic hyphaema treated with the antifibrinolytic drug tranexamic acid *Acta ophthal (Kbh)* 54 250–256

Bramsen T (19) Traumatic hyphaema treated with the antifibrinolytic drug tranexamic acid II *Acta ophthal (Kbh)* 5, 616–620

Bramsen T (19 8) A double blind study on the influence of tranexamic acid on intraocular pressure and the central corneal thickness after trabeculectomy for glaucoma simplex *Acta ophthal (Kbh* (in press)

Bramsen T Corydon L & Ehlers N (1978) A double blind study of the influence of tranexamic acid on the central corneal thickness after cataract extraction *Acta ophthal (Kbh)* 56 121–126

Bramsen T & Ehlers N (197) Bullous keratopathy (Fuchs endothelial dystrophy) treated systemically with 4 trans amino cyclohexano carboxylic acid *Acta ophthal (Kbh)* 55 665–6 3

Bramsen T & Stenbjerg S (19 9) Variations in serum antifibrinolytic activity following eye operations *Acta ophthal (Kbh)* (in press)

Ehlers N (19 4) Graft thickness after penetrating keratoplasty *Acta ophthal (Kbh)* 52 893–903

Ehlers N & Sperling S (19) A technical improvement of the Haag Streit pachometer *Acta ophthal (Kbh)* 5 333–336

Mishima S (1968) Corneal thickness *Surv Ophthal* 13 57–96

Rammer L & Saldeen T (1970) Inhibition of fibrinolysis in post traumatic death *Thromb Diath haemorrh (Stuttg)* 24 68–75

Remé C & Witmer R (1974) *Absence of fibroplasia following wounds of the primate iris* In International symposium on wound healing Rotterdam 19 4 The Foundation International Cooperation in the Medical Sciences Montreux 1975

Ygge J (19 0) Changes in blood coagulation and fibrinolysis during the postoperative period *Amer J Surg* 119 225–232

Author's address

Dr T Bramsen
Department of Ophthalmology
Århus Kommunehospital
DK 8000 Århus C
Denmark

Department of Ophthalmology (Head N Ehlers)
Århus Kommunehospital University of Aarhus Denmark

HUMAN CORNEAL ENDOTHELIUM IN ORGAN CULTURE

The Influence of Temperature and Medium of Incubation

BY

STEFFEN SPERLING

Forty four human corneas from patients between 21 and 86 years were incubated in Eagle s minimum essential medium with Earle s salts 10–46 h post mortem The influence of incubation temperature and composition of the medium on endothelial survival was evaluated Whole corneas were stained by alizarine red Recent cell loss was indicated by morphological alterations in the endothelial pattern After 20–28 h of incubation minimum cell loss was found at 31 C when 8 % Dextrane 250 and 20 % serum or 8 % Dextrane 500 and 10 % serum was added to the medium

Key words alizarine red – dextrane – endothelium – human cornea – morphology – temperature – organ culture

his study was undertaken when it was realized that the endothelium on hole human corneas incubated in organ culture under conditions as described y Summerlin et al (1973) showed signs of continuous cell death (Sperling 378) The aim of the present study was to evaluate the influence of varied icubations procedures Evaluation was based on recognition of morphological lterations in the endothelial cell pattern after cell loss

Normal endothelial cells form joint meetings of three Each cell is sur ounded by 4–8 neighbouring cells The borders between neighbouring cells ieet at angles approximating 120 Pointed angles are extremely rare During ncubation in tissue culture medium damaged cells are expelled from the oherent cell sheat by expanding neighbours Four or more expanding cells orm joint meetings In minutes to hours these cells slide and form joint

Received August 15 1978

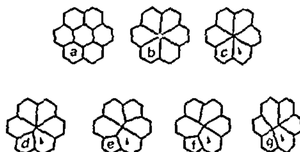


Fig 1

a–c Formation of joint meeting of six originally hexagonal cells d–g Transform[illegible] to joint meetings of four cells Arrows indicate *reformation figure centres*

meetings of three or four During reformation of joint meetings of three cells appear in characteristic patterns shown in principle in Figs 1 d g (Sherrard 1976 Olson et al 1978 Sperling 1978)

In each pattern Figs 1 d g two or more cells with converging pointed [illegible] or one cell with a pointed angle plus a joint meeting of four occur A [illegible] of cells appearing in one of these configurations (*in the following term*[illegible] *reformations figures*) was regarded as indication of recent loss of one ce[illegible]

Material and Methods

Whole human eyes were enucleated from cadavers stored at 15–22 C for 8–10 h [illegible] later at 10 C Hypotonic eyes with indented corneas or with contact between [illegible] cornea were rejected. After rinsing in tap water corneas were excised with a 1.5 [illegible] rim of sclera, and the endothelium was stained for one min by sterile 0.5% trypan blue in 0.9% NaCl Forty four corneas in which less than one per cent [illegible] endothelial cells were found in unfolded areas (Sperling 1978) were suspended *100 ml* glass infusion bottles by a suture through the scleral rim and incubated [illegible] *25 ml of* tissue culture medium (Fig 2) Patient age and time between death [illegible] incubation is indicated for each cornea in *Fig 3*

The basic medium was composed of Eagle's minimum essential medium with Ear[illegible] salts L-glutamine (0.092 mg/ml) $NaHCO_3$ (2.2 mg/ml) amicasin (Bristol 0.1 [illegible] carbenicillin (Astra 0.5 mg/ml) and nystatin (Squibb 50 IU/ml) Ten or 20 per cent foetal calf serum (Microbiological Ass) and Dextrane (Pharmacia) with mean [illegible] cular weights 50 000 250 000 or 500 000 were added to the medium. The bottles [illegible] inflated with a gas of 95 per cent air and five per cent CO and placed a[illegible] temperature



Fig 2

. excised cornea suspended by a suture through the scleral rim in 95 ml of medium in a 100 ml glass infusion bottle

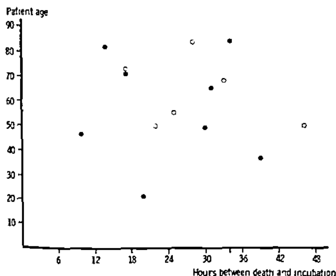


Fig 3

atient age and time between death and incubation indicated for each of the forty our cadaver corneas included in this study An open circle represents one cornea A dot represents two corneas from the same patient.

After 20–28 h of incubation the corneas were rinsed briefly in minimum essential medium and the endothelial cell borders were stained for four min by 0.1% alizarin red 10% saccharose in deionized water (Sperling 1977) Stained whole corneas were placed epithelium down on microscope slides and filled by minimum essential medium. The flattened central 6 mm of the endothelium was studied in a light microscope fitted with a photo tube Objective Zeiss 10× Film Polaroid 667 85×105 mm.

The microscope was focused at random in the central flattened area and photographs were obtained of each cornea In the experiments on temperature dependence a photograph was obtained when 3/4 or more of a full microscopic field could be brought in focus Some folding of the membrane of Descemet was present at the beginning of the experiments Increased corneal thickness during incubation in media without Dextrane led to progressive endothelial folding Many reformation figures appeared on the slopes of endothelial folds and in rows parallel to the folds. These reformation figures were excluded from the estimates of cell damage by the procedure of test area selection In experiments with Dextrane in the medium less folding occurred and the microscope was focused at random in the central area. On each photograph one small and three large frames were drawn The total number of cells was counted in one frame equivalating 0 030 mm on cornea and the number of reformation figure centres were counted in three frames each equivalating 0 0 6 mm on cornea *Centres* of reformation figures were determined as indicated by arrows in Fig 1 The frames were drawn and the numbers of cells and reformation figure centres per frame were counted according to the rules described by Sperling & Gundersen (1978) By this procedure the total number of cells were counted in areas per cornea and the number of reformation figure centres were counted in twelve areas Counts from the same cornea were averaged and the number of reformation figures were expressed in per cent of the number of cells per area.

Results

Twenty corneas were incubated in the basal medium with 10% calf serum at temperatures between 19 C and 37 C (indicated temperature ± 1 C). In Fig 4 the percentages of reformation figures after 20–28 h of incubation are related to temperature Minimum cell damage occurred between 25 C and 34 C The cellular morphology after 20–28 h of incubation at 37 C and 31 C is illustrated in Figs 6 & 7

In the preceeding experiments increase in the corneal thickness during incubation lead to progressive endothelial folding In four corneas addition of 5% Dextrane 50 to the medium reduced but did not abolish the progressive folding Four corneas were incubated at 31 C with eight per cent Dextrane 250 added to the medium Slight endothelial folding was still present after incubation but reformation figures did not accumulate in relation to folds on these corneas Folding was minimal in four corneas incubated with eight per cent Dextrane 500

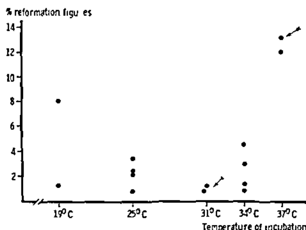


Fig 4

ormation figures after 20–28 h of incubation in per cent of the total number of s per area related to incubation temperature Arrows indicate corneas shown in Figs 6 & 7

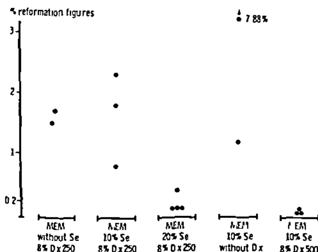


Fig 5

formation figures in per cent of the total number of cells per area after 20–28 h incubation in Eagle s minimum essential medium with Earle s salts (MEM) foetal calf serum (Se) and Dextrane (Dx) at 31°C.

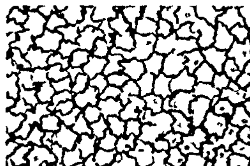


Fig 6

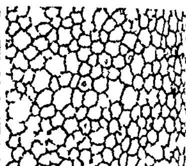


Fig 7

Fig 6 Endothelial cell borders stained by alizarine red after 23 h of incubat 37 C From the cornea marked with a single arrow in Fig 4 Magnification 135

Fig 7 Endothelial cell borders stained with alizarine red after 96 h of incuba 31°C From the cornea marked with a double arrow in Fig 4 Magnification 1

The percentages of reformation figures after 20–23 h of incubatio in the basal medium without serum with 10 per cent serum with 20 per c serum without Dextrane with Dextrane 250 and with Dextrane 500 are dicated in Fig 5 Four corneas were included in each of these experim Less than one per cent of reformation figures was found when 20 % se and 8 % Dextrane 250 or 10 % serum and 8 % Dextrane 500 was added the medium

Comments

In this study corneas were prepared for incubation 10–46 h post mortem T endothelium was extremely sensitive to mechanical trauma during enucleati and preparation of the corneas Most eyes with post mortem times of than 36 h were rejected because of corneal indentation Only corneas w less than one per cent blue stained endothelial cells were incubated It app from Fig 3 that patient age and post mortem time were not correlated T indicates that the state of the endothelium can not be predicted from post m tem time from patient age or from a combination of the two

An earlier study (Sperling 1978) indicates that a complete reformation the endothelial cell pattern occurs within hours after cell loss If this observ tion is correct the percentage of reformation figures will be related to actu endothelial death rate and independent of initial cell density and initial damage left undected by trypane blue staining

he present study indicates that an incubation temperature at or below temperature *in vivo* is essential for endothelial survial *in vitro* (Fig 4) llel observations were made on rat oral mucosa and epidermal cells *in* ı (Jepsen 19/4 Indo & Wilson 197/)

ı a basic medium comparable to the medium used in the present study trane 40 was found to be toxic to rabbit endothelium at 3/ C (Geeraets et 977) and to human endothelium at 25 C (Sachs et al 1978) In the present y the least amount of cell damage was found when eight per cent of trane 2ɔ0 or Dextrane 500 was added to the medium of incubation at] Eight per cent was an arbitrary choice Experiments on prolonged bation in media containing Dextrane are in progress They may show ther the observed positive effect of Dextrane is merely an initial pheno on caused by a lower initial swelling rate

Acknowledgments

author wishes to express his gratitude to Birte Olesen Department of Ophthal ɔgy Århus Kommunehospital for skilled technical assistance and to Inger Søren MD Statens Seruminstitut Department in Århus for advise on the bacteriological ɔlems encountered during this study This study was supported by a grant from Danish Committee for Prevention of Blindness Dextrane was kindly supplied by DA A/S Copenhagen and amicasin by Novo Industries A/S Copenhagen.

References

raets R McLester W D & McMullan F D (1977) Corneal endothelium under arious storage conditions *Amer J Ophthal 83* 206–212

o K & Wilson B R (1977) Foetal rat keratinizing epidermal cells in culture ffects of long term treatment by Benzo(a)pyrene on their growth characteristics *nat Cancer Inst 59* 867–874

sen A (19/4) An *in vitro* model of a keratinizing squamous epithelium *Scand J Dent Res 8*9 144–146

on L E Marshal J Rice N S C & Andrews R (19/8) Effects of ultrasound on he corneal endothelium II The endothelial repair process *Brit J Ophthal 6*9 45–154

hs U Goldman K Valenti J & Kaufman H E (1978) Corneal storage at room emperature *Arch Ophthal (Chicago) 96* 1075–1077

errad E S (19/6) The corneal endothelium *in vivo* Its response to mild trauma *Exp Eye Res* 99 347–3ɔ7

erling S (19/) Combined staining of corneal endothelium by alizarine red and rypane blue *Acta ophthal (Kbh) 5ɔ* ɔ73–580

Sperling S (19/8) Early morphological changes in organ cultured human corneal endothelium *Acta ophthal (Kbh) 56* /85–792
Sperling S & Gundersen H J (19/8) The precision of unbiased estimates of numerical density of endothelial cells in donor corneas *Acta ophthal (Kbh)* 56 93...
Summerlin W T Miller G E, Harris J E & Good R A (19 3) The organ-cultured cornea An in vitro study *Invest Ophthal 1°* 1/6–180

Author's address
Steffen Sperling
Department of Ophthalmology
Aarhus Kommunehospital
DK 8000 Aarhus C
Denmark

From the Department of Ophthalmology (Head Salme Vannas)
University of Helsinki

CORNEAL ENDOTHELIAL CELL DENSITY IN IRIDOCYCLITIS

BY

KIRSI SETÄLÄ

The endothelial cells of both eyes of 60 unilateral iridocyclitic patients were photographed with a specular microscope to establish the possible effect of iridocyclitis on corneal endothelial cells
Chronic severe iridocyclitis with mutton fat keratic precipitates (KP) lowered the central endothelial cell count Five patients out of the seven with mutton fat KPs had a distinctly lower central cell density in the affected eye In the remaining patients no significant difference in cell densities could be demonstrated between affected and healthy eyes Neither the inflammatory process itself nor the round white KPs had a deleterious effect on the central corneal endothelial cell densities

Key words iridocyclitis – keratic precipitates – corneal endothelial cell density – endothelial cell loss – clinical specular microscope

e corneal endothelium is a delicate tissue which is susceptible to changes in environment Specular microscopy provides a mean for direct photographic imination of the *in vivo* corneal endothelium at relatively high magnifica ns

In an earlier paper (Setala & Vannas 1978) we reported the difference in corneal endothelial cell counts between the affected and healthy eyes of sner Schlossman patients The main factor affecting the endothelial cell nsity seemed to be the intermittently high IOP (Vannas et al 1977) The ier possible detrimental factors in the Posner Schlossman patients were kera precipitates (KP) and aqueous changes This led us to study iridocyclitic es to elucidate the effects of KPs and aqueous changes on endothelial cell nsity

ceived September 28 1978

Farlier studies of enucleated eyes revealed some differences of opini/ cerning the effect of iridocyclitis on corneal endothelial cells Stocker assumed that the inflammatory process as such had a deleterious effect on endothelium and Irvine (1956) observed localized flattening and atten of the endothelium under KPs

In the present study we examined and photographed both eyes of 6 lateral iridocyclitic patients with a specular microscope and compar endothelial cell counts between eyes of the same patients

Material and Methods

The series comprised 60 patients in the Department of Ophthalmology U versity of Helsinki (Fig 1) There were 32 women and 28 men The a the patients ranged from 11 to 74 years with a mean of 41 4 years E *patient had unilateral iridocyclitis with no history of inflammation* in other eye which was externally and internally normal The contral healthy eye served as control Both eyes had normal intraocular pressure underlying aetiologic disease was ankylosing spondylitis in six cases rheu toid arthritis in six cases and sarcoidosis in two cases One patient had Re syndrome In the remaining cases the aetiology was unknown (Table I

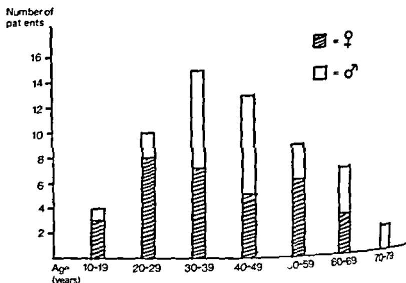


Fig 1
Age and sex distribution of patients

-ents were divided into three groups according to their signs and symptoms ble II A B C) (Hogan et al 1959) Seven patients had mutton fat keratic cipitates 35 had round white KPs and the third group (18 patients) dis -yed no definite KPs during the examinations

'he endothelial cells were photographed with a specular microscope Several ures five on average were taken of the central cornea of both eyes The phology size and cell density were analyzed and counted as described in previous paper (Vannas et al 1977)

Table 1

Aetiology of iridocyclitis	
Ankylosing spondylitis	6
Rheumatoid arthritis	6
Sarcoidosis	2
Reiter s syndrome	1
Others	45
	60

Table 2 A
Patients with mutton fat KPs

Sex	Present age/age at onset (years)	Recurrences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm)		
					affected eye	control eye	difference (%)
m	49/48	3 x	++	+	2401	2981	+19 5
m	15/12	chronic	+	–	3064	3519	+15 4
m	22/20	chronic	+	–	2650	3105	+14 7
f	34/24	> 10 x	++	–	2650	3064	+13 5
f	49 26	chronic	++	+	2484	2815	+11 8
f	11/9	chronic	+	–	3188	3271	+ 2.5
m	50 48	5 x	++	+	1987	1987	0
							+11 % mean

Table 2 B

Patients with small or medium sized round white KPs

Sex	Present age/age at onset (years)	Recurrences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm)		
					affected eye	control eye	differ.
f	27/26	3 ×	++	–	2950	3146	[illegible]
f	59/40	2 ×	+	–	2950	3100	+49
f	28/23	> 10 ×	++	–	3090	3730	+40
m	63/52	5 ×	++	+	2836	2939	[illegible]
m	31/28	> 10 ×	++	+	2740	2370	[illegible]
m	40/38	3 ×	+	–	2567	2650	+30
f	26/26	no recurrences	+	–	2980	3040	[illegible]
f	31/28	3 ×	+	–	2788	2836	+1
m	48/45	> 10 ×	++	+	2732	2770	+14
f	29/27	3 ×	+	–	3105	3146	+13
m	49/40	6–7 ×	++	+	2492	2443	[illegible]
m	32/[illegible]	6 ×	+	–	2732	2770	+05
f	22/22	no recurrences	+	–	2770	2790	+0
f	65/60	chronic	+	–	2980	2980	0
m	51/43	> 10 ×	++	+	2150	2150	0
m	67/58	6–7 ×	++	+	2898	2898	0
m	24/18	5 ×	++	–	2939	2939	0
f	55/52	5 ×	+	–	2627	2627	0
m	74/[illegible]	2 ×	++	+	2815	2815	0
f	24/22	2 ×	++	+	3105	3105	0
m	44/42	3 ×	+	–	2815	2815	0

cont.

Results

The endothelial cell densities of the healthy and affected eyes are presen- in Table II A B and C

Seven patients had a chronic severe iridocyclitis with only intermitten- quiescent periods These eyes had mutton fat KPs Five of these patients dis- played a distinctly lower central endothelial cell density in the affected eye

Table 2 B (cont)

Present age/age at onset (years)	Recurrences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm²)		
				affected eye	control eye	difference (%)
40/40	no recurrences	++	–	2981	2981	0
57/57	no recurrences	++	–	3105	3105	0
40/39	no recurrences	++	+	2650	2650	0
32/32	no recurrences	++	+	2931	2931	0
62/48	6 x	++	–	2194	2194	0
73/73	2 x	++		2120	2120	0
36/34	3 x	+	–	3015	3015	0
45/?	> 10 x	+	–	3312	3312	0
32/30	8 x	++	–	2788	2770	–0 6
52/49	2 x	++	–	2608	2567	–1 0
31/31	no recurrences	+	–	3353	3312	–1 2
31/?	6–7 x	++	–	2900	2860	–1 4
3 /30	5–6 x	++	–	3022	2980	–1 4
66/?	> 10 x	++	+	2730	2690	–1 5
patients						+0 7 % mean

the healthy eye The mean IOP of the affected eyes was 12 mmHg and of control eyes 14 mmHg

The second group of patients had round white KPs The cell density was ver in the iridocyclitic eye of 13 patients but the same in both eyes of 16 tients In six eyes the endothelial cell density was slightly lower in the althy than in the iridocyclitic eyes The mean difference was 0 7 % The an IOP was 13 mmHg in the affected eyes and 16 mmHg in the control es

Table 2 C
Mild iritis with no discovered KPs

Sex	Present age/age at onset (years)	Recurrences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm)		
					affected eye	fellow eye	differ[illegible] (%[illegible]
f	44/44	no recurrences	+	–	2650	2730	+3[illegible]
m	34/34	no recurrences	+	–	2677	2732	[illegible]0
f	51/50	3 x	+	–	2318	2360	+15
m	49 49	no recurrences	+	–	2401	2443	+17
f	43/42	> 10 x	+	–	2480	2480	0
f	22/18	2 x	+	–	2900	2900	0
f	18/?	2 x	+	–	2732	2732	0
f	25/19	2 x	++	–	3146	3146	0
m	34/31	no recurrences	++	+	2815	2815	0
f	16/16	no recurrences	++	+	3395	3395	0
m	44/44	no recurrences	+	–	2732	2732	0
f	30/30	no recurrences	++	+	2950	2950	0
m	36/36	no recurrences	++	+	3684	3684	0
f	62/62	no recurrences	+	–	2150	2150	0
f	64/64	no recurrences	++	+	2291	2263	12
f	34/33	3 x	++	–	31[illegible]0	3120	16
f	59/51	3 x	+	–	20[illegible]0	2029	[illegible]0
f	59/57	4 x	++	–	3064	2931	[illegible]9
18 patients							+0.04 mean

n the third group of 18 patients no KPs were seen at the time of photo phy and only a few small KPs or debris had previously been noticed on endothelial surface Only four patients had a lower cell density in the ected eye Ten patients had the same cell density in both eyes and four had ower cell density in the healthy control eye The mean IOP was 12 mmHg he affected eyes and 15 mmHg in the control eyes

These three groups are rather small precluding statistical comparison An eresting result is that in the first group of patients with mutton fat KPs severe iridocyclitis 71 % of the affected eyes had a lowered cell density npared with the healthy eyes In the second group with white KPs there s some tendency towards a lower cell density in the affected eyes (+0 7 %) the third group in which no KPs were detected no difference could be wn between affected and healthy eyes

rrelation with duration of disease

venteen patients had had only one attack of 1–3 weeks in duration These ients were photographed at the end of the attack No clear difference was iced between eyes of these patients

Twenty nine patients had had two or more iridocyclitic attacks before otography Only four of these patients had a history of less than two years iritis and in the remaining 25 patients two or more years had elapsed be een the first attack and the time of photography A small tendency towards vered endothelial cell density was discovered in these patients None of the ients showed a clear difference between healthy and affected eyes

Fourteen patients had a chronic disease or several recurrences with only ort healthy intervals Twenty nine per cent of these patients had distinctly ver cell counts in the affected eyes The lowered endothelial cell densities rrelated well with mutton fat KPs every patient with a clearly lower cell nsity also had mutton fat KPs

orphology of the endothelial cells

e diseased eyes with a lower cell density had large cells and showed a more egular cellular pattern than the control eyes which showed a regular mosaic e pattern (Fig 2)

Discussion

atients with mutton fat KPs displayed a lowered endothelial cell count in e affected eye in 71 % of the cases Round white KPs or the inflammatory rocess per se did not seem to influence the central corneal endothelial cell

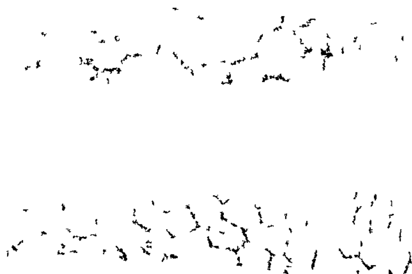


Fig 2
Large somewhat hazy irregular endothelial cells in iridocyclitic eye with mu ton KPs (A) compared with the normal endothelium of the healthy fellow eye of th patient (B) ×350

density These results were obtained by photographing the central cor endothelial cells of 60 unilateral iridocyclitic patients with a specular mic scope and comparing the healthy and affected eye of the same patients P vious investigators have found the cellular pattern to be similar in both e of normal individuals Kaufman et al (1966) examined 180 normal pati and noted that 98 % had an identical endothelial cell pattern in both e Various patients in this study received differing therapeutic regimens but particular mode of therapy could be specially correlated with endothelial c loss

A major limitation of the method is that only a small area of the end thelium is photographed with each exposure Further the same area cannot found later and our method does not allow us to select areas under KPs photography Areas under the KPs could be seen only hazly and could not well documented It is our impression that KPs by covering the endothel cells inhibit the cell reflexes that are photographed by the endothelial came That is why the exact cell number and cellular survival under the KPs can be determined

ı cell loss takes place ın the lower part of the cornea ıt should be reflected the cell count of the central area because healıng occurs by slıdıng rather n by cell multıplıcatıon (Bourne & Kaufman 19/6 Kaufman & Katz 19/6) ı another study Blackwell et al (19//) compared central endothelıal cell mbers wıth perıpheral cell counts No dıfferences were found between cen tl superıor temporal and ınferıor areas of the cornea (except ın theır ıntra ılar lens group) One less studıed varıable ıs the change ın relatıve cell den ıes wıth tıme *No ınformatıon ıs avaılable concernıng* the length of tıme red after a profound loss of endothelıal cells ın one area before other cells ead and equalıze cell counts ın the affected and perıpheral areas In our v 16 patıents were photographed after theır fırst ırıdocyclıtıc attack but the remaınıng cases enough tıme had elapsed for endothelıal cell spread to ur before photography

The patıents wıth mutton fat KPs dısplayed a lowered endothelıal cell count the affected eye Thıs ıs ın agreement wıth prevıous studıes ın whıch epıthelıal lema was often notıced ın areas correspondıng to KPs (O Connor 19/2) tenuatıon and localızed flattenıng of the endothelıum under KPs has also n observed ın long standıng ırıdocyclıtıs (Irvıne 1956)

Inomata & Smelser (19/0) produced uveıtıs by ıntravıtreal ınjectıon of bo ıe serum albumın ınto albıno rabbıts The ınflammatıon caused corneal ckenıng and hazıness Ultrastructural endothelıal changes ıncluded mono clear cell ınfıltratıon and endothelıal vacuolızatıon Many of these ınvadıng ls were located between the endothelıum and Descemet s membrane but endothelıal cell cytoplasm showed lıttle evıdence of change It ıs espe ılly noteworthy that the apıcal junctıonal complexes of the endothelıal cells uld easıly open to admıt ınvadıng cells and reform after these cells had tered The greatest changes ın the endothelıum occurred ın the area of pre ıtates

Based on the above ıt appears that temporary endothelıal damage and func ınal changes may occur ın experımental ırıdocyclıtıs of relatıvely short dura ın ın rabbıts but actual cell loss ıs not a general fındıng These studıes agree th our clınıcal fındıngs Patıents wıth recurrent attacks of nongranulomatous eıtıs dıd not show ınterocular dıfferences ın endothelıal cell densıtıes be een healthy and affected eyes In contrast patıents wıth mutton fat keratıc ecıpıtates and chronıc dısease showed dıstınctly lower central endothelıal cell nsıtıes ın theır affected eyes

References

Blackwell W L Gravenstein N S & Kaufman H E (1977) Comparison of central corneal endothelial cell numbers with peripheral areas Amer J Ophthal 473–476

Bourne W M & Kaufman H E (1976) Specular microscopy of human corneal endothelium in vivo *Amer J Ophthal 81* 319–323

Hogan M J Kimura S J & Thygeson P (1959) Signs and symptoms of uveitis *Amer J Ophthal 47* pt. II 155–171

Inomata H & Smelser C (1970) Fine structural alterations of corneal endothelium during experimental uveitis *Invest Ophthal 2* 2–78.5

Irvine A R, Jr (1956) The role of endothelium in bullous keratopathy *Arch Ophthal (Chicago) 56* 338–351

Kaufman H E Capella A & Robbins J E (1966) The human corneal endothelium *Amer J Ophthal 61* 835–841

Kaufman H E & Katz J I (1976) Pathology of the corneal endothelium *Nei Eye Institute Symposium on Experimental Pathology* Bethesda Md., 1976

O Connor G R (1972) Calcific band keratopathy *Trans amer ophthal Soc* 57–81

Setala K & Vannas A (1978) Endothelial cells in the glaucomato-cyclitic crisis *Ophthal 36* 218–224

Stocker F W (1971) *The Endothelium of the Cornea and Its Clinical Implications* 2nd ed. Thomas Springfield Ill

Vannas A, Setala K & Ruusuvaara P (1977) Endothelial cells in capsular glaucoma *Acta ophthal (Kbh) 55* 951–958

Author's address

Kirsi Setala
The University Eye Clinic,
University of Helsinki
00290 Helsinki 29
Finland.

Department of Ophthalmology (Head V Ehlers)
Århus Kommunehospital University of Aarhus Denmark

PRIMARY LOCALIZED ORBITAL AMYLOIDOSIS

BY

JENS HENRIK NEHEN

The present paper reports a case of primary localized amyloid tumour of the orbit in a 65 year old woman in whom myasthenia gravis had been diagnosed 33 years before An amyloid tumour with this location is very rare, and up to the present time only 13 cases have been recorded in the literature The reported orbital amyloid tumour revealed a considerable accumulation of activity at orbital scintigraphy with $^{99}Tc^{m}$ and compared with the conventional pseudotumours a distinct rise in density at com puted tomography after intravenous infusion of iodized contrast medium

Key words amyloidosis – orbital amyloidosis – orbital tumour – myasthe nia gravis – $^{99}Tc^{m}$ scintigraphy – computed tomograpny

nyloid is a fibrous protein which is collected in the ground substance between e cells and fibres It most notably involves the walls of capillaries and arte oles and the surrounding tissues The most useful histological test for amyloid staining with Congo red It stains collagen and elastic tissue as well as amy id but all forms of amyloid regardless of its location reveal a green di iroism and birefringence when viewed in a polarizing microscope

The main amyloid protein is a micro fibre which possesses properties distinct om those of any other known mammalian protein These include a charac ristic appearance on electron microscopy and a characteristic X ray diffrac on pattern with the fibrils consisting of polypeptide chains arranged in an ntiparallel conformation with a β pleated structure (Glenner et al 1973)

There is no general agreement as to the best classification of the various

eceived June 15 1978

Table 1

	Sex	Age (years)	Duration of history	Proptosis	Limitation of ocular movements	Localiza[illegible]
Pollems (1920)	♀	42	2	+right –left	+right –left	behind both the s[illegible] and the inferior orbit[illegible] margin of the right o[illegible] and behind the super[illegible] orbital margin of th[illegible] orbit
Handousa (1934)	♂	45	1	+	+	upper and inner qu[illegible] of the left orbit
Easton et al (1961)	♀	49	4	+	+	inn[illegible]r part of the rig[illegible] orbit outside the m[illegible] cone
Gronowski et al (1965)	♀	3[illegible]	5	+	+	lower and outer qua[illegible] of the left orbit
Howard (1966)						
case 1	♀	2[illegible]	2	–	+	the region of the left lacrimal gland
case 2	♂	60	4	+	+	upper and inner qua[illegible] of the right orbit
Kassman et al (1967)	♀	53	12	+	+	behind the inferior or[illegible] margin of the right or[illegible]
Raab (1970)	♂	55	9	+	+	behind the left superior orbital margin and in upper and inner quadr[illegible] of the right orbit
Radnot et al (1971)	♀	62	<1	–	–	the region of the right lacrimal gland
Schubert (1972)	♀	[illegible]8	[illegible]	+	–	lower and outer quadr[illegible] of the right orbit
Knowles et al (1975)	♀	61	2	–	–	the regions of both lacrimal glands
Jensen (1976)	♀	49	[illegible]	+	+	inner parts of both orb[illegible]
Savino et al (1976)	♂	51	1	+	+	upper and outer quadr[illegible] of the right orbit
Nehen (1978)	♀	65	2	+	+	behind the superior orb[illegible] margin of the left orb[illegible]

Table 1

Consistence	Attached to bone	Lymphocytes	Plasma cells	Foreign body giant cells	Electron microscopy	Amyloid infiltration of vessel walls	Partial excision	Total excision	Recurrence	Capsule	Positive anti nuclear factor
hard	+	+	+	+		+	+right		+		
soft	+	+		+		+	+			–	
hard	+	+	+	+		+	+		+	–	
hard		+	+	+		+	+		+	–	
	+			–		+		+			
hard				+		+	+		+	–	
hard	+					+	+		–		
hard			+			+	+left			–	
					+	+		+			
hard						+		+			
hard		+	–		+	+	+				
hard		+	+	+	+	+		+right			
hard		+	+	+			+				+
hard	+	+	+	+		+	+		+	–	+

manifestations of amyloidosis The one suggested by Reimann et al. (classified amyloidosis as primary when there was no underlying disease secondary when there was an associated chronic disease as tumour for when small localized masses of amyloid were found and as amyloidosis associated with multiple myeloma in the presence of the latter disease This generally remained as the major clinically operational classification.

Primary localized orbital amyloidosis appears to be extremely rare and lated case reports account for the principal literature The pathogenesis is known and the relationships to the other patterns of amyloidosis are unc

Up to the present time only 13 cases of primary localized orbital amyloid have been recorded in the literature These cases are surveyed in Table I an additional case is presented

Case Report

A 68 year old woman in whom myasthenia gravis was diagnosed in 1944 has been under treatment with neostigmine In 1946 she received X ray irradiation thymus (3000 r) In 1948 right sided maxillary and frontal sinusitis was treated osteotomy of the right maxilla In 1971 resection of the right maxillary sinus performed it was filled with fibrous tissue which did not contain amyloid. In a marked swelling around the left eye suddenly developed it was accompanied moderate impairment of vision which has persisted unchanged afterwards. After time the patient had recurrent episodes of pain and lacrimation in the left eye

In 1974 at the age of 65 years the patient was treated for the first time Department of Ophthalmology University Hospital Århus Pronounced pr (Hertel exophthalmometer right eye 12 mm left eye 21 mm) downward displ slight ptosis loss of upward movement of the left eye and diplopia were noted the temporal two thirds of the supraorbital margin a hard immobile tumour palpated it extended posteriorly between the orbital roof and the eyeball and to the underlying bone Ophthalmoscopy revealed a slight impression and a f the upper half of the retina caused by compression by the tumour Visual ac eye 1 0 left eye 0 4 Incipient cataract of the left eye Applanation tonometry eye 12 mmHg left eye 17 mmHg No visual field defects

Tomography of the left orbit showed blurring of the soft structures but no destruction or hyperostosis Left sided orbitophlebography disclosed block of the rior orbital vein anteriorly in the medial part of the orbit Orbital $^{99}Tc^{m}$ scan showed a large accumulation of activity upwards in the left orbit (Fig 1)

Partial excision was performed through an anterior orbitotomy with the a piece of tissue measuring 1 × 2 5 × 3 cm from the anterior part of a large greyish red slightly bleeding tumour situated between the orbital roof and the

Histologically the tumour was made up of nodules of an amorphous eosino material which showed a strongly positive reaction to amyloid staining with Co and distinctly dichroism in polarized light The eosinophilic material was sh up by collagen fibres Scattered in the tissue were fairly large lymphocyte infiltra

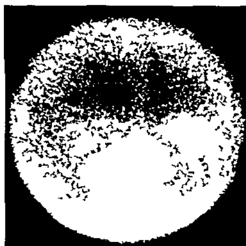


Fig 1
rbital $^{99}Tc^m$ scintigram Large accumulation of activity upwards in the left orbit

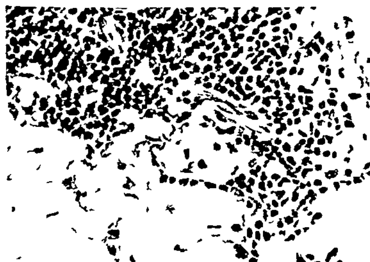


Fig 2
nyloid deposits with scattered foreign body giant cells and in the connective tissue many lymphocytes and a few plasma cells Haematoxylin and eosin × 300

and a smaller number of plasma cells At the margins of the amyloid areas, fo- foreign body giant cells were present (Fig 2)

Biopsy specimens from the liver and rectal mucosa showed no signs of am- Immuno electrophoresis showed reduced amounts of IgA Antinuclear fa- positive with medium reaction

The erythrocyte sedimentation rate had been elevated since 1971 and was th- at the time of operation

Laboratory tests and clinical examination gave no basis for either systemic pr- or secondary amyloidosis

During the next few years periods with increasing protrusion and limitation of movements occurred In 1977 tomography of the left orbit showed thinning of orbital roof and as before density of the soft tissue of the upper half of the or- Biopsy revealed an amyloid tumour of the same character as before In 1978 the p- trusion of the left eye had increased to the same extent as before the first excision a visual acuity was unchanged No Bence Jones protein was present in the urine C- puted tomography disclosed a tumour superiorly and laterally in the left orbital co- it was 1 cm in width and extended 2 cm posteriorly There were exostosis and a m- speckled osseous structure of the lateral orbital wall at the level of the tumour. density values in the tumour tissue were between 43 and 51 Hounsfield units be- *the injection of the iodinated contrast medium and between* 60 and 80 units after injection (Fig 3) No treatment had been given

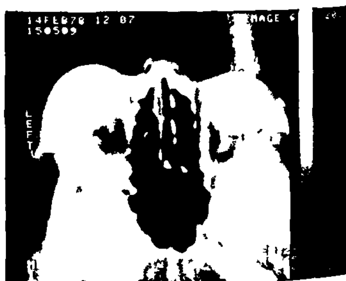


Fig 3

Computed tomography A tumour superiorly and laterally in the left orbital ar- with exostosis of the lateral orbital wall and protrusion of the left eye (after contrast infusion)

Discussion

le I gives a survey over the reported cases of primary localized orbital loidosis

he ages of the 13 previously described patients (9 women and 4 men) ranged 1 27 to 78 years Only four cases were bilateral Apart from the lacrimal s the clinical picture was characterized by slowly progressing proptosis and tation of eye movements often associated with diplopia With a single ex ion the tumours were hard and – in all cases in which information was lable firmly attached to the underlying bone and without a capsule

ommon to all the cases is a histological picture with partial replacement of connective tissue by irregularly shaped aggregations of amyloid material in most of the cases marginal infiltration with foreign body giant cells arterioles invariably showed amyloid infiltration of their walls In almost of the cases there was infiltration of plasma cells and lymphocytes in the nective tissue In only a few of the cases had total excision been possible out recurrence These include all the amyloid tumours located in the imal gland In the cases where the amyloid tumours were large with ex ion deeper into the orbit and with more infiltrative growth there was a ked tendency to recurrence and repeated local excision had often been ired

he cause of amyloidosis is not known Several pathogenic mechanisms have considered through the years

he role of immune mechanisms in the genesis of amyloid is a topic that has widely discussed and has resulted in a varity of opinions

lenner et al (1973) are of the opinion that the amyloid fibrils are formed n immunoglobulin proteins They have demonstrated that the major pro i component of the amyloid fibrils both in cases of primary systemic amy losis and in isolated tumours composed of amyloid is an immunoglobulin it polypeptide chain and/or its amino terminal fragment

he role of the reticulo endothelial system in the pathogenesis of amyloid also received considerable attention According to experimental studies of lum (1964) amyloidosis is the result of a persistent antigenic stimulation mesenchymal tissue leading to a break down or a perversion of the protein thesizing function of the reticulo endothelial system His two phase cellular ory of the formation of amyloid consists of a pre amyloid phase marked by increased proliferation of pyroninophilic reticular and lymphoid cells The ond phase is the actual amyloid phase characterized by a suppression of proli ating pyroninophilic mesenchymal cells and the appearance of PAS positive ls These cells secrete an abnormal protein deposited locally as amyloid

Although the significance of the immune apparatus in the pathoge... experimental amyloidosis can be considered an established fact, the w... which its functional state may influence the development of amylo... clear The role of the thymus was studied by Ranlov (1966) He showed in mice previously subjected to thymectomy or combined thymectomy and lethal irradiation experimental caseinate amyloidosis developed more ra... and to a greater extent than in control animals He concluded that thymu... tends to promote the development of amyloid disease perhaps through th... mination of a superior function of the thymus in regulating the protein the is of mesenchymal tissue In the absence of the thymus even short l... antigenic stimulations can be imagined to lead to a break down of the m... chymal tissue function with subsequent deposition of amyloid In Ranlov... vestigations immuno electrophoresis was not employed but Arnason (1964) reported a subnormal level of IgA in thymectomized mice

The cases reported by Groniowski et al (1965) and Jensen (1966) were... *cussed in the light of Teilum s two phase cellular theory* of the form... amyloid and in both the two phases could be observed Furthermore in J... sen s case the presence of many IgG carrying lymphoid cells could s... Glenner s theory of the immunoglobulin origin of amyloid Both were of opinion that their cases could be a reaction to some immunological p... perhaps an auto immune reaction

In the present case it is noteworthy that the patient had for many y... uffered from myasthenia gravis which is generally considered an auto-im... disease In view of Ranlov s work on the role of thymectomy in the deve... ment of amyloidosis it is also of interest that the patient had been treated w... irradiation of her thymus

The reported orbital amyloid tumour revealed a considerable accumula... of activity at orbital scintigraphy which also can be demonstrated in s... pseudotumours (Heuer & Ehlers 1972) but compared with the conventi... orbital pseudotumours there was a distinct rise in density at computed to... graphy after intravenous contrast infusion

According to Wackenheim et al (1977) the density of a pseudotumour computed tomography is practically unchanged after intravenous infusi... iodized contrast media On the other hand Gyldensted et al (1977) rep... a rise in density values corresponding to an average of 14 4 Hounsfield u... Before contrast infusion the density of orbital pseudotumours does not d... essentially from several other orbital tumours and a differential diagnos... not possible

The present paper is the first in which a description of both computed to... graphy and orbital scintigraphy of an orbital amyloid tumour is given, which reason it is not possible for any comparison to be made

References

-rason B G St Cyr C. de V & Shaffner J B (1964) A comparison of immuno lobulins and antibody production in the normal and thymectomized mouse. *Immunol 93* 915–925

ton J A & Smith T H (1961) Non specific granuloma of orbit (orbital pseudo imour) *J Path Bact 82* 345–354

nner G G., Terry W D & Isersky C (1973) Amyloidosis Its nature and patho enesis *Sem Hematol 10* 65–86

mowski J Bernardczykowa A. & Norn M S (1965) Orbital amyloidosis *Acta phthal (Kbh) 43* 725–729

ldensted C., Lester J & Fledelius H (19 7) Computed tomography of orbital lesions *Neuroradiology 13* 141–150

ndousa A (1954) Localized intra-orbital amyloid disease. *Brit J Ophthal 38* 10–311

uer H E & Ehlers N (1972) L Orbitographie avec le Technetium 99m dans le diagnostic des tumeurs orbitaires *Ann d'Oculist (Paris) 205* 283–290

ward G M (1966) Amyloid tumours of the orbit. *Brit J Ophthal 50* 421–425

nsen O A. (19 6) Bilateral amyloidosis of the orbit *Ophthalmologica 173* 0–78

ssman T & Sundmark E (1971) Orbital pseudotumours with amyloid *Acta ophthal Kbh) 45* 270–273

rowles D M., Jacobiec F A Rosen M & Howard G (19 5) Amyloidosis of the orbit and adnexa *Surv Ophthal 19* 367–384

llems W (1970) Über tumorformige lokale Amyloidosis in der Orbita *Graefes Arch Ophthal 101* 346–359

ab E. L. (19 0) Intraorbital amyloid. *Brit J Ophthal 54* 445–449

ldnot M Lapis K & Feber J (19 1) Amyloid tumour in the lacrimal gland. *Ann Ophthal 3* ? –742

nley P (1966) The role of the thymus in experimental mous amyloidosis *Acta path microbiol scand 67* 4 –54

eimann H. A., Kouchy R. F & Eklund C. M (1935) Primary amyloidosis limited to tissue of mesodermal origin. *Amer J Path 11* 9 –988

ssino P J Schatz N J & Rodriques M M (19 6) Orbital amyloidosis *Canad J Ophthal 11* 252–255

chubert E (19 2) Amyloidtumor der Orbita *Klin Mbl Au enheilk 160* 467–468

eilum G (1964) Pathogenesis of amyloidosis The two phase cellular theory of local secretion. *Acta path microbiol scand 61* 91–45

Wackenheim A., Damme W van Kosmann P & Bittighoffer B (19) Computed tomography in ophthalmology *Neuroradiology 13* 135–138

Author's address

H Nehen,
Department of Ophthalmology
Arhus Kommunehospital
DK 8000 Arhus C.
Denmark

From the Eye Department (Head P Brændstrup)
Kommunehospitalet Copenhagen

POSTMORTAL "VITAL' STAINING OF THE EXTERNAL EYE

BY

M S NORN

Two hundred and two eyes from 112 individuals were vital stained from 2 to 46 h after death Intensity and extension of staining were studied in ten regions The staining gradually progressed after death also within non exposed areas It most often started anteriorly on the tarsus and finally included the fornix

Microscopy revealed diffuse cell staining by rose bengal or trypan blue The cell nucleus was most often more intensely stained than the cytoplasm Up to five h after death neutral red had only stained vacuoles in the cytoplasm Later diffuse staining of cells occurred

Tetrazolium differed from the above dyes in that the pronounced staining seen immediately after death gradually decreased in the course of time Microscopy disclosed stained inclusion bodies in the cytoplasm up to 15 h after death Then cell staining was only seen as a rare exception in relation to extracellular dye granules However the mucous thread in the inferior fornix showed gradually increasing postmortal tetrazolium staining The amount of mucus was found to be the same in dead persons as in the living

The characteristic appearance of the dead eye is due among other things, to ruptures of the corneal epithelium (fluorescein stained) and cell death and not to drying up or coating by mucus

Key words cornea – conjunctiva – vital staining postmortal double rose bengal – trypan blue – neutral red – fluorescein – tetrazolium iodonitrotetrazolium – alcian blue

Received June 26 1978

heading of this paper Postmortal Vital Staining may seem self con dictory Strictly speaking we cannot *vital stain dead* persons However the ls take longer to die than the individual as such and cell death extends to ferent regions at different intervals from the moment of death

The eyes change in appearance after death They become glazed Film oducers wanting to give a perfect illusion of death can do so by instilling k into the conjunctival sac

The object of the present study has been to clarify the superficial changes ermining the postmortal appearance Are they due to mucous coatings de hment of corneal epithelium drying up or putrefraction? Where and when the changes start?

The eyes were vital stained and studied in slit lamp and microscope at dif ent postmortal stages The technique employed approximated that used in vious examinations of living eyes (Norn 1974)

Method

rior to vital staining I noted down whether the dead person s eye was closed open and if open the width of the opening in millimetres

Then the eye was further opened and three dye drops of 0 01 ml each were tilled on the corneal centre as well as superiorly and inferiorly on the lb The dye drop now flowed into the superior and the inferior fornix The e was thereafter distributed by blinking carried out by the examiner oving the lids several times Finally the eye was washed with 2–3 ml of a lanced saline solution (4 9 mg NaCl 0 75 mg KCl 0 48 mg CaCl 0 3 mg gCl in 1 ml)

The following dyes were employed A mixture of 1 % fluorescein and 1 % se bengal or a mixture of 1 % iodonitrotetrazolium and 1/4 % alcian blue or % tryptan blue or 1 % neutral red (Norn 1974)

The vital staining was studied in Kowa s hand held slit lamp magnifying times After staining with tetrazolium five min were allowed to elapse be ore examination in order to ensure enzyme reaction

The results were graded from *1* to *5* (*1* indicating only few dots (< 30) < 100 3 < 1000 4 < 10 000 and 5 > 10 000 dots) The results for the ten amined regions of each eye were entered in a diagram (Fig 1)

Specimens for microscopy were prepared by scraping with a platinum atula from conjunctiva or cornea or by transferring a mucous thread from e inferior fornix to a glass slide between two small wooden sticks Each ecimen was kept in a moist chamber For microscopy the glass slide was

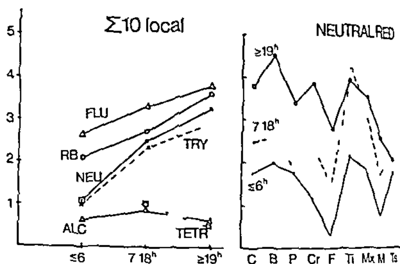


Fig 1

Left diagram Mean staining grade of ten regions at different intervals after d. FLU (fluorescein) assessed on the cornea only RB (rose bengal) NEU (neutral TRY (trypan blue) ALC (alcian blue) TETR (tetrazolium)
Right diagram Mean staining grade of nine regions at different intervals after d. C (cornea) B (bulb) P (plica) Cr (caruncle) F (inferior fornix) Ti (inferior tar Mx (Marx line) M (mucous thread) Ts (superior tarsus)

covered by a cover slip holding one drop of balanced saline solution. Microscopy of the specimens stained by tetrazolium and neutral red for which time interval was critical followed within one h The other specimens tolerated storing for up to 24 h

The statistical calculation was performed using Mann Whitney s rank sum test unpaired data

Material

A total of 202 eyes from 112 individuals with no diagnosed eye disease were examined Mean age 72 (ranging from 36 to 91) median 73 (10 and 90 percentile 57 and 86 years) 47 per cent were females

The dead bodies were kept in cellars (temperature about 10-14 C) and examined before being transferred to the postmortem room

Vital staining was performed from 2 to 46 h after death in 45 cases within 6 h in 80 between 7 and 18 h and in 77 after not less than 19 h

Microscopy was undertaken of 108 scrapings and 26 mucous threads from 57 individuals equally distributed over the different vital staining groups

Results

e eyes of normal living persons only become stained to a very small extent the vital stains employed (Norn 1972a 1974) As a general rule the vital ining was found to increase in intensity and extension the longer the inter l after death Exceptions were tetrazolium and alcian blue (Fig 1 left dia am and Table I) The staining was independent of age and sex

orescein

ins epithelial defects being therefore rarely seen in normal eyes

The first three postmortal h a moderate punctate staining was noticed spread t over the whole cornea (on an average grade 1 8) but after four h a con intense punctate staining was seen to cover the cornea (grade 3 5 $<$0 05) After 10 h the great majority and after 20 h the total number of es also displayed an extensively stained erosion This was located centrally ing often transversely oval and comprising up to one third of the corneal ea

Increasing punctate staining was also seen on the conjunctiva

The fluorescein staining and the erosion were independent of whether the e had been open or closed postmortally

Table I

ostmortal vital staining Mean staining grades at short and longer intervals after ath assessed for five regions (h hours after death) N number of eyes tarsus inferior tarsus n s non significant)

Vital stain	h	N	Cornea	Bulb	Fornix	Tarsus	Mucous thread
Neutral red	≦ 6	19	1 75	2 00	0 95	2 17	1 08
	> 6	24	3 21	3 83	2 95	4 17	2 13
	P		- 0 01	< 0 01	< 0 01	⩽ 0 01	< 0 01
Rose bengal	≦ 6	8	1 63	3 13	0 38	1 75	1 50
	> 6	34	3 76	4 02	2 47	3 62	1 59
	P		⩽ 0 01	< 0 05	⩽ 0 01	⩽ 0 01	n s
Trypan blue	≦ 6	12	1 00	1 33	0 00	0 75	1 50
	> 6	50	2 66	3 26	1 54	3 60	1 96
	P		⩽ 0 01	⩽ 0 01	⩽ 0 01	⩽ 0 01	= 0 05
Tetrazolium	≦ 18	41	1 56	1 15	0 68	1 32	0 88
	> 18	21	0 86	0 24	0 10	0 14	0 14
	P		< 0 05	< 0 05	< 0 05	⩽ 0 01	< 0 01

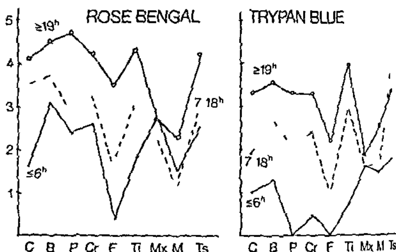


Fig 2

Left diagram Postmortal vital staining of nine regions with rose bengal
Right diagram Vital staining with trypan blue
Abscissa regions abbreviated as in Fig 1 right

Trypan blue

stains dead cells

The staining was modest within the first six h after death It was most pr-
nounced over the areas of the prekeratinized line along the lid (M ex ler-
and the mucous thread which both contain many dead cells This is also the
case in living persons (Norn 1967)

The staining increased during the next h so much so that after more tha-
19 h all regions were stained to a degree no less than Marx line (right dia-
gram Fig 2)

Soon after death the cornea showed punctate staining forming a horizont-
band Several h later such staining most often covered the whole cornea

Soon after death the bulb was stained at about 3 and 9 o clock and late
also at about 6 o clock After 19 h the staining extended all the way ro-
the limbus corneae

Staining of the tarsus most often started anteriorly at the margin les f-
quently posteriorly (20 against 9 cases) Late examination showed the sta-
to have spread over the whole tarsus in most cases

The staining was independent of whether the eye was opened or closed

Microscopy Scraping and mucous threads showed zones with stained cells whose nu-
were more intensely stained than their cytoplasm

_le bengal

ns degenerate cells death cells and mucus In normal eyes of living persons y Marx line caruncle and mucous thread become intensely stained whereas other regions are only slightly stained (Norn 1974)

The staining was seen to increase gradually in all regions after death reach a maximum everywhere by the late postmortem examination (left diagram 2 Table I) The staining seemed generally to be more extensive than er trypan blue

The staining was not like keratoconjunctivitis sicca staining localized within exposed area and it was independent of any closing defect On early imination universally spread punctate staining was seen over the cornea ending to the bulb above and below the cornea but rarely temporally nasally The triangular exposed area might even remain unstained unlike surrounding areas More than 18 h after death all the examined eyes owed dense punctate staining of most of the cornea and bulbar conjunctiva nerally round the entire limbus) spreading to the fornix and the tarsus e staining of the superior tarsus most often started anteriorly

croscopy Most of the cells from scrapings and mucous thread presented universally ead staining in some instances nuclei more so than cytoplasm

utral red

ins inclusion bodies in the cytoplasm of the cells of living persons (Marner Norn 1978)

Slit lamp examination revealed a gradually increasing staining the longer e interval after death The grade and distribution of the staining corre onded approximately to that for trypan blue (Fig 1) The staining grade as less than that obtained with rose bengal The staining independent of an en or closed eye most often started anteriorly on the tarsus

icroscopy The first four h after death red staining was only observed in inclusion dies in the cytoplasm the same as in living persons From five h after death I also ticed weak diffuse staining of odd cells the same grade in nucleus and cytoplasm fter six h increasing diffuse cell staining occurred with the result that inclusion dies were no longer discernible After seven h the staining of nuclei was more in nse than that of cytoplasm The number of stained cells increased After 18 h in nsely stained regions were seen in which practically all the cells were involved – ist as in a well stained specimen prepared for histological examination

This development included both granulocytes and all species of epithelial cells

The vital staining observed within the first five h after death represented a reaction living cells (stained inclusion bodies) After seven h all the stained cells were dead nes

This microscopical finding is in keeping with the fact that neutral red stain greater extent than trypan blue within the first six postmortal h while subsequ the staining grade corresponds to that of trypan blue (compare right diagram F with right diagram Fig 2 neutral red stains 86 % trypan blue 59 % of 170 local < 6 h $t = 4.3$ $P < 0.001$)

Tetrazolium

rarely stains normal living eyes and if it does the staining is low grade, cor nea in 5 per cent tarsus in 10 per cent Marx line in 5 per cent Norn 19

As early as two to six h after death pronounced punctate staining was not only of the exposed cornea but also of the non exposed bulb tarsus a fornix The staining of the cornea either formed a horizontal band or was versally spread In rare cases there was intense staining of flakes or ere The staining of the superior tarsus was most often spread over the whole more rarely concentrated anteriorly and very rarely posteriorly alone T colour faded the longer the interval from the moment of death The reacti to tetrazolium thus contrasted with that to the above dyes The inferior f was not stained after 21 h and the other areas not after 26 h

The mucous thread in the inferior fornix was nearly always stained (1 cent) and its red colour grew increasingly intense the longer the postmo period in other words a reaction contrasting definitely with those of the o regions

Microscopy Until 18 h after death red inclusion bodies were found in epithelial of scrapings and mucous thread The stained cells were concentrated in zones, often 50 to 80 per cent of the cells contained red inclusion bodies The cells had sorbed the colourless tetrazolium and reduced this to red formazan in organelle the cytoplasm (Norn 1974)

After 19 h no inclusion bodies were visible Nests of red granules occu red extracellularly in the mucous thread Occasional epithelial flakes having red such nests of dye granules were diffusely red with nuclei and cytoplasm equally sta The pronounced local accumulation of formazan had evidently caused staining of cells as in a specimen for histological examination

Alcian blue

is a specific mucus staining dye Mucus is always present in the living no eye in the fornix Punctate staining of mucus may in some instances als seen on the bulbar conjunctiva the tarsus Marx line the caruncle and th more rarely the cornea (Norn 1963 1972b)

Fig 3 shows the mean staining grade in dead persons being ird pe of the interval after death Punctate staining of mucus on the cornea was in 37 per cent and on the bulbar conjunctiva in 23 per cent not differen nificantly from the living eye

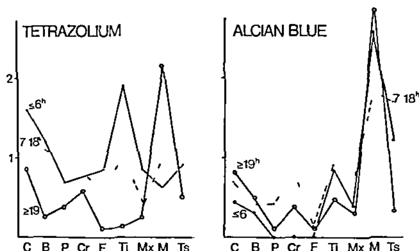


Fig 3

tmortal staining with iodonitrotetrazolium (left diagram) and alcian blue (right diagram) Abscissa regions abbreviated as in Fig 1

A mucous thread is always present in the inferior fornix Double staining th tetrazolium and alcian blue gave a mainly red mucous thread in 34 per it suggesting bacterial infection (Norn 1974) and a mainly green one in remaining cases

icroscopy Extracellular mucous fibrils were seen in nearly all scrapings In the mu is thread all the mucous fibrils had an intense green colour

In a few exceptional cases odd green stained cells were seen from 9 h after death ter 18 h zones of such were present in 5 per cent where the nucleus was more in nsely stained than the cytoplasm

Discussion

he results of the present investigation confirmed that cell death sets in later ian the individual s death and that the cell death proceeds to different re ions at different points of time after death A kind of topography of death he present investigation gave results suggesting that cell death most often arts anteriorly on the superior tarsus and reaches the fornix long after the ther regions

The glazed eyes (the altered appearances of cornea and bulbar conjunc tiva after death) are hardly due to drying up the alterations being inde

pendent of open or closed eye from the moment of death Further the r bengal stained area was not identical with the exposed one On the co the exposed triangles of bulbar conjunctiva might remain unstained the surrounding area and the tarsus became intensely stained

The postmortal changes were *not* due to deposits of mucus becase *amount of mucus does not increase after death and the mucous thread* mains unchanged in the inferior fornix

The glazed eyes were caused by increasing cell death (the cells were by trypan blue neutral red and rose bengal) and corneal ruptures (pur and erosions stained by fluorescein)

The time of death can roughly be estimated by vital staining one er neutral red for instance and the other with tetrazolium and then stud the result in slit lamp and microscope (Figs 1 and 3) Note however th considerable differences may be seen with regard to grade and extension the staining between the two eyes of the same dead persons (Grade 1 c ference in 11 7 % grade 2 in 3 3 % grade 3 in 0 3 % no difference in 84 – 1200 *localities*)

With neutral red the staining increases postmortally The reverse is the c with tetrazolium the latter only staining cells whose enzyme function is p served However the mucous thread in the fornix shows increasing tetraz staining evidently because extracellular *detritus likewise may produce* r granules on an enzymatic basis

References

Marner K & Norn M S (1978) Neutral red *Acta ophthal (Kbh)* 56 47–730

Norn M S (1963) Mucus on conjunctiva and cornea *Acta ophthal (Kbh)* 41 13

Norn M S (1967) *Trypan blue vital staining of cornea and conjunctiva* *Acta oph (Kbh)* 45 380–389

Norn M S (1972a) Vital staining of cornea and conjunctiva (fluorescein rose be mixture and tetrazolium alcian blue mixture) *Acta ophthal (Kbh) Suppl* 113 pp

Norn M S (1972b) Tetrazolium alcian blue mixture I *Acta ophthal (Kbh)* 50 5

Norn M S (1974) *External Eye Methods of Examination* pp 199 Scriptor Copenha

Author s address

Mogens Norn M D
Eye Department
Københavns Kommunes Hospital i Hvidovre
Kettegård Alle 30
DK 2650 Hvidovre
Denmark

Department of Ophthalmology (Head Sven Erik G Nilsson)
University of Linkoping Sweden

THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

III The Bulbar Zone the Zone of the Fornix and the Supranodular Zone

BY

STEFAN LATKOVIC

Three adjacent zones of the conjunctival epithelium of the guinea pig are described the three layered bulbar zone with cuboidal superficial cells the three layered zone of the fornix with cylindrical superficial cells and the four to five layered supranodular zone characterized by close association with a lymphoid nodule The similarities and differences in the general morphology of the epithelium and in the ultrastructural features of the cells as compared to those of the perilimbal zone are pointed out The functional significance of atypical mitochondria found in certain cells of the bulbar and fornical zones is commented upon Particular attention is paid to the supranodular zone containing a large number of intraepithelially located lymphocytes The possible role of an epithelium associated with subepithelial lymphoid tissue in the immune defense is discussed

Key words conjunctival epithelium – bulbar zone – zone of the fornix supranodular zone – atypical mitochondria – lymphocytes – guinea pig ultrastructure – transmission electron microscopy

e morphology of the basal intermediate (Latkovic & Nilsson 1979a) and erficial (Latkovic & Nilsson 1979b) layers of the perilimbal zone of the nea pig conjunctival epithelium was described in preceding papers The sent study concerns the ultrastructure of the bulbar zone the zone of the

eived July 6 19 8

pendent of open or closed eye from the moment of death Further the r bengal stained area was not identical with the exposed one On the co- the exposed triangles of bulbar conjunctiva might remain unstained w the surrounding area and the tarsus became intensely stained

The postmortal changes were *not* due to deposits of mucus because amount of mucus does not increase after death and the mucous thread mains unchanged in the inferior fornix

The glazed eyes were caused by increasing cell death (the cells were st- by trypan blue neutral red and rose bengal) and corneal ruptures (p- and erosions stained by fluorescein)

The time of death can roughly be estimated by vital staining one eye r neutral red for instance and the other with tetrazolium and then stu- the result in slit lamp and microscope (Figs 1 and 3) Note however t considerable differences may be seen with regard to grade and extens- the staining between the two eyes of the same dead persons (Grade 1 d- ference in 11 7 % grade 2 in 3 3 % grade 3 in 0 3 % no difference in 8[illegible] – 1200 localities)

With neutral red the staining increases postmortally The reverse is the c with tetrazolium the latter only staining cells whose enzyme function is p- served However the mucous thread in the fornix shows increasing tetra- staining evidently because extracellular detritus likewise may produce granules on an enzymatic basis

References

Marner K & Norn M S (1978) Neutral red *Acta ophthal (Kbh)* 56 [illegible]

Norn M S (1963) Mucus on conjunctiva and cornea *Acta ophthal (Kbh)* 41 13-

Norn M S (1967) Trypan blue vital staining of cornea and conjunctiva *Acta o- (Kbh)* 45 380–389

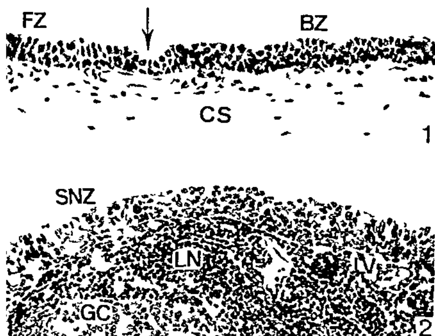
Norn M S (1972a) Vital staining of cornea and conjunctiva (fluorescein rose ben- mixture and tetrazolium alcian blue mixture) *Acta ophthal (Kbh) Suppl* 113 pp

Norn M S (1972b) Tetrazolium alcian blue mixture I *Acta ophthal (Kbh)* 50

Norn M S (1974) *External Eye Methods of Examination* pp 199 Scriptor Copenha-

Author s address

Mogens Norn M D
Eye Department
Københavns Kommunes Hospital i Hvidovre
Kettegård Alle 30
DK 2650 Hvidovre
Denmark



Figs 1 and 2

ow power light micrograph of the conjunctival epithelium showing the bulbar zone and the zone of the fornix (FZ) Note the rather uniform thickness of the epi um The conjunctival stroma (CS) contains only a few cells To the right of the w are seen the cuboidal superficial cells of the bulbar zone To the left of the w many of the cells are cylindrical which is typical of the zone of the fornix)

ow power light micrograph of the supranodular zone (SNZ) of the conjunctival elium LN lymphoid nodule GC germinal centre LV lymph vessel ×340

ı in the corresponding cells of the perilimbal zone Thus no dark and ht cells were found The vesicular and vacuolar apparatus of the cells in intermediate and superficial layers was not as well developed and the intra ular cysts in the superficial cells were infrequent

he microfilaments were less numerous in the intermediate and superficial s Melanosomes were absent throughout all layers In some of the inter liate and superficial cells the common mitochondria were replaced for the t part by large and strangely shaped bodies presumably atypical mito ndria (Fig 3)


MV
S
CY
S
S
P
N
AM
AM
N
P
Mi

zone of the fornix

een in survey light (Fig 1) and electron micrographs (Fig 4) the epithe
ı of the fornical zone was predominantly three layered The basal cell layer
similar to that of the bulbar zone The intermediate cell layer did not con
light and dark cells of the types found in the perilimbal zone but some
hedral cells presumably transitional forms between the basal and super
ıl cells showed a less electron dense cytoplasmic matrix than the other ones
ımpanied by a decreased number of microfilaments and an increased number
esicles and vacuoles

.s a marked difference between the bulbar and fornical zones the super
ıl cells of the latter zone were cylindrical in shape except at both peripheries
the zone where they were more cuboidal The cytoplasmic matrix of the
erficial cells was less electron dense than that of the basal and most inter
liate cells and the microfilaments were fewer and more dispersed Numerous
ochondria and a moderate number of vesicles and vacuoles were present
ge coalesced vacuoles were not observed however and the intracellular
s were fewer than in the perilimbal zone

ertain superficial and a limited number of intermediate cells in all other
ctural respects identical to other cells of the same layer contained a large
ıber of strangely shaped bodies presumably atypical mitochondria (Figs 4
. 5) Very few common mitochondria were observed in these cells The
pical mitochondria showed great variations in both shape and size including
ıe rather bizarre forms It seems that different planes of sectioning con
uted to the great polymorphism seen in the micrographs No serial sections
e cut

n the sections the atypical mitochondria hardly ever showed cristae of the
al type but instead profiles of what appeared to be saccular or tubular struc
es sometimes concentrically arranged Many of them enclosed rounded seg
nts (vacuoles) with a content similar to or slightly less electron dense than
cytoplasmic matrix In several cases this segment was found to be continuous
h the cytoplasm of the cell (Figs 4 and 5) These arrangements made some
the mitochondria appear ring shaped or crescent shaped respectively

Fig 3

ey electron micrograph of the intermediate polyhedral cells (P) and superficial
oidal cells (S) of the epithelium of the bulbar zone An intracellular cyst (CY) is
monstrated Two of the superficial cells contain atypical mitochondria (AMI)
N nucleus MI *mitochondria* I *interdigitations* MV *microvilli* x 8750


VA
MI
CL
CL
AM
GO
N
N
AM
D
LY
P
P
N
B
B

f the specialized cells a moderate number of goblet cells were observed in superficial layer increasing towards the supranodular zone of the epithe l (Fig 4) Small lymphocytes (Fig 4) occurred with moderate frequency could be found in all cell layers of this zone Plasma cells were rarely nd and then only in the basal and intermediate cell layers

supranodular zone

ymphoid nodule is located subepithelially near the base of the lid at about of the distance between the limbus and the lid margin It consisted of densely ked lymphoid cells with darkly stained nuclei except in the germinal cen where the cells had a lighter appearance (Figs 2 and 6) The population lymphocytes was very heterogeneous showing morphological variations from all to medium sized lymphocytes with numerous intermediate forms Isolated sma cells and reticular cells were also seen Small lymph vessels were dis buted along the periphery and a few inside the nodule

The overlying conjunctival epithelium was separated from the lymphoid dule by the basal lamina (*Figs 6 and 7*) *In light micrographs of sections* ined with silver nitrate the basal lamina appeared to be discontinuous Elec n micrographs showed that at the points where the basal lamina was absent oups of lymphocytes were traversing it (Fig 7)

Inside the epithelium the lymphocytes were most often observed as small oups located in all layers (Figs 6 and 8) The cell membrane was smooth d no junctions between lymphocytes or between lymphocytes and adjacent ithelial cells were seen (Fig 8) The intraepithelial lymphocytes were pre minantly of the medium sized type with variations mainly in the nuclear to toplasmic ratio and the quantity of cytoplasmic organelles The shape of the cleus followed the shape of the cell (Fig 8) Single ribosomes and polyribo mes were dispersed throughout the cytoplasm The large Golgi complex ve

---

Fig 4

arvey electron micrograph of the epithelium of the zone of the fornix Above a yer of basal cells (B) a single intermediate layer of polyhedral cells (P) is en. The superficial layer consists of cylindrical cells (CL) one of which contains great number of atypical mitochondria (AMI) A goblet cell (GO) is interposed etween the cylindrical cells A small lymphocyte (LY) is located in the intermediate yer N nucleus MI mitochondria VA vacuole I interdigitations F bundles of microfilaments Arrow points to the basal lamina x 6500



Fig 5

Atypical mitochondria with cristae in form of tubules or saccules in a superficial [illegible] of the zone of the fornix. Some of the profiles are ring shaped or crescent-like [illegible] encircling cytoplasm with ribosomes and vesicles. Arrows point to the place where the surrounded part of the cytoplasm is continuous with the cytoplasm of [illegible]

VE small vesicles, VA vacuole, R ribosomes, N nucleus. x 57 6[illegible]



Fig 6

...vey electron micrograph of the epithelium of the supranodular zone and a part ...the lymphoid nodule with lymphocytes some of which can be identified as medium ...ed lymphocytes (MLY) Three intraepithelial islets of aggregated lymphocytes (LY) ...seen. The basal cells (B) appear flattened and the polyhedral cells (P) of the ...ermediate layers are rather irregular in shape The microvilli at the free surface ...the superficial cells (S) are uneven and sometimes strangely shaped Arrow points to the basal lamina VA vacuole MI mitochondria × 3500



Fig 7

A group of lymphocytes (LY) is seen partly in the lymphoid nodule and partly in the epithelium The basal lamina is absent at this point A polyhedral cell (P) of the intermediate layer is separated from a basal cell (B) by a lymphocyte (LY) Arrows point to the basal lamina × 13 100

s vacuoles mitochondria some multivesicular bodies and long profiles of rough surfaced endoplasmic reticulum were usually found concentrated in rtain section of the cell compartment Structures resembling secondary osomes were observed in some lymphocytes

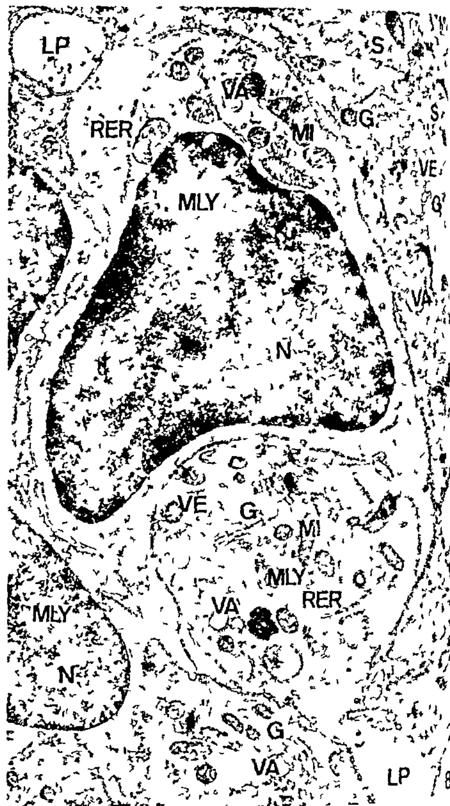
he conjunctival epithelium overlying the lymphoid nodule consisted of four ive distorted cell layers The irregularities in the arrangement of the cell rs and in the shape of many of the cells was caused by the islets of invading phocytes The basal cells were flatter than those of the previously described s As to ultrastructural aspects the basal cells and the polyhedral cells in intermediate layers resembled the corresponding cells in the fornical zone melanocytes and no melanosomes were observed The superficial cells con ed a moderate number of microfilaments Large compound vacuoles and acellular cysts were absent On the free cell surface the microvilli showed it variations in shape and incidence In other respects the cells in the super il layer were similar to those of the perilimbal zone

'ery few goblet cells were observed and only at the periphery of the supra ular zone

Discussion

ny of the general ultrastructural characteristics such as the presence and ribution of intracellular components described and discussed for the three n epithelial cell types (basal intermediate and superficial cells) of the peri bal zone of the conjunctival epithelium of the guinea pig (Latkovic & Nils 1979a b) were also typical for the three zones of the epithelium covering bulb the fornix and the lymphoid nodule Differences seen even at low gnification concerned the number of cell layers (three in the bulbar zone and zone of the fornix and four to five in the perilimbal and supranodular es) the cell shape (cylindrical cells in the superficial layer of the zone of fornix) and the cell types (melanocytes were absent whereas small num s of goblet cells were observed in all zones described in the present paper) Certain cells in the superficial layer and less often in the intermediate layer the bulbar zone and the zone of the fornix contained a large number of what peared to be atypical mitochondria Such atypical mitochondria were not erved in the other zones of the conjunctival epithelium

As seen in the micrographs profiles of several atypical mitochondria re nbled partly open rings Similar structures in the interstitial cells of rat testis ristensen & Chapman 1959) and in the cells of rat liver (Stephens & Bils 55) were previously described under the name of cup shaped mitochondria both reports the authors pointed out the increased contact surface between


LP
S
VA
RER
MI
G
S
VE
MLY
G
VA
N
VE
G
MI
MLY
RER
VA
MLY
N
G
VA
LP
8

mitochondrion and the cytoplasm facilitating the exchange of metabolic mediates as well as the possibility that the enclosed cytoplasm may be dified enzymatically Cup shaped mitochondria were also observed by Rosen m et al (1969) in alveolar cells of oxygen adapted and poisoned rats The ors suggested that the increased surface to volume ratio might represent a npensatory response to diminished enzymatic activity Similar mitochondria re reported for the lutein cells of the rat corpus luteum actively secreting gesterone (Enders & Lyons 1964) Other forms of atypical mitochondria observed in the adrenal cortex of hamster (De Robertis & Sabatini 1958 es 1965) mouse (Zelander 1959) rat (Sharawy & Penney 1973) and in renocortical cells in culture (Suyama et al 19//) Many of these authors re the atypical mitochondria as one of the signs of active steroidogenesis The question as to what the functional significance of the atypical mitochon a in the conjunctival epithelium might be and why they occur only in cer n cells of the bulbar and fornical zone and not in any other parts of the epi ium cannot be answered at the moment

The anatomical arrangement of an epithelium overlying a lymphoid tissue described earlier for the intestinal tract (appendix Peyer s patches in the all intestine) and for the oropharynx (lingual palatine pharyngeal tonsils) e e g Rhodin 1974) Subepithelial aggregations of lymphocytes in the human njunctiva some of which resemble lymphoid nodules were reported among by Norn (1960) and by Kessing (1968) The only mention of the sub njunctival lymphoid tissue in the guinea pig to our knowledge was made by Master et al (1967) and Tenner et al (1971)

Characteristic for the association of an epithelium and lymphoid tissue is the ding of a great number of intraepithelial lymphocytes aggregated in small oups In the cases of the oropharyngeal and intestinal tracts it is generally ed that the lymphocytes migrate from the lymphoid tissue into the over ng epithelium The basal lamina and the intercellular junctional complexes parently do not hinder the movements of the lymphocytes through the epi ium According to Steer (1975) the tissue boundaries are no barrier to the

Fig 8

dium sized lymphocytes (MLY) located near the free surface of the superficial lls (S) Intracellular organelles such as mitochondria (MI) Golgi complex (G) cles (VE) vacuoles (VA) and profiles of rough surfaced endoplasmic reticulum ER) are concentrated at one pole of the lymphocyte The nucleus (N) is located at opposite pole The cell membrane is smooth without interdigitations and inter cellular junctional complexes LP lymphocyte process MV microvilli × 16 300

migration of the cells from the tissue of mesodermal origin through [illegible] endodermal origin The fate of the intraepithelial lymphocytes has [illegible] tively been elucidated Some authors (Faulk et al 1971 Rhodin [illegible] 1975) favour a theory that they leave the epithelium via the free surfa[illegible] others (Meader & Landers 1967 Glaister 1973 Bjerregaard 19[illegible]) [illegible] the lymphocytes return to the subepithelial lymphoid tissue The rather [illegible] number of lymphocytes found in human tear fluid (Norn 1960) su[illegible] former theory In our material we could not find decisive signs suppo[illegible] one of these theories

While the lymphoid nodule contained a heterogeneous population of [illegible] cytic cells the lymphocytes observed inside the epithelium all had some [illegible] characteristics also indicating an active cell a large Golgi complex [illegible] creased number of mitochondria and of free ribosomes polyribosomes [illegible] profiles of rough surfaced endoplasmic reticulum In the lymphoc[illegible] line these cells are known as "intermediate" or medium sized [illegible] (Zucker Franklin 1969 Rhodin 1974)

Similar cells were found in the bursa of Fabricius (Clawson et al 1[illegible] Peyer s patches of rabbit intestine (Faulk et al 1971) in the intestinal [illegible] lium of the mouse (Glaister 1973) and in the intestinal epithelium of the [illegible] (Bjerregaard 19[illegible]) It was suggested by the above mentioned authors [illegible] as by Fichtelius (1967) that intraepithelial lymphocytes and subepithelial [illegible] phoid tissue participate in immune responses According to Bjerregaard [illegible] an immunologically competent small lymphocyte can be stimulated by [illegible] transported through the epithelial cells Depending on the nature of th[illegible] either cellular immunity is triggered or the stimulated cell differentia[illegible] an IgA synthesizing plasma cell In the course of this progress the small [illegible] phocyte passes through stages in which it is indistinguishable from the [illegible] mediate lymphocyte (Rhodin 1974)

Acknowledgment

This investigation was supported by grants from the Swedish Medical Researc[illegible] (Project No 12X 734) and the Research Committee of the Ostergotlands Lans [illegible] ting

References

Bjerregaard P (197[illegible]) Lymph[illegible]id cells in chicken intestinal epithelium *Cell T[illegible]* *161* 48[illegible]–4[illegible]

Carroll J M & Kuwabara T (19[illegible]8) Ocular pemphigus *Arch Ophthal* (Chic[illegible] 683–69[illegible]

stensen A K & Chapman G B (19ɔ9) Cup shaped mitochondria in interstitial lls of the albino rat testis *Exp Cell Res 18* ɔ16–579
wson C C Cooper M D & Good R A (1967) Lymphocyte fine structure in the ırsa of Fabricius the thymus and the germinal centers *Lab Invest 16* 40ı–4°1
Robertis E & Sabatini D (19ɔ8) Mitochondrial changes in the adrenocortex of ɔrmal hamsters *J biophys biochem Cytol 4* 667–678
ders A C & Lyons W R (1964) Observations on the fine structure of lutein cells I The effects of hypophysectomy and mammotrophic hormone in the rat *J Cell I °? 1°ı 141*
ılk W P McCormick J N Goodman J R Yoffey J M & Fudenberg H H 19 1) Peyer s patches morphologic studies *Cell Immunol 1* ɔ00–ɔ?0
htelius K E (1967) The mammalian equivalent to bursa Fabricii of birds *Exp ell Res 46* °31–°34
uster J R (19ı3) Light fluorescence and electron microscopic studies of lymphoid lls in the small intestinal epithelium of mice *Int Arch Allergy 4ɔ* 8ɔ4–86ı
in° S V (1968) *Mucous gland system of the conjunctiva A quantitative normal ıatomical study* pp ı6–87 *Acta ophthal (Kbh) Suppl 9ɔ*
c S (19ı9) The ultrastructure of the normal conjunctival epithelium of the nea pig IV The palpebral and the perimarginal zones *Acta ophthal (Kbh)* in press)
tkovic S & Nilsson S E G (19ı9a) The ultrastructure of the normal conjunctival pithelium of the guinea pig I The basal and intermediate layers of the perilimbal ne *Acta ophthal (Kbh) ɔı* 106–1°2
tkovic S & Nilsson S E G (19ı9b) The ultrastructure of the normal conjunctival pithelium of the guinea pig II The superficial layer of the perilimbal zone *Acta ophthal (Kbh) ɔ7* 123–13ɔ
tkovic S & Nilsson S E G (19ı9c) Phagocytosis of latex microspheres by epi helial cells of the conjunctiva *Acta ophthal (Kbh)* Submitted for publication
aster P R B Aronson S B & Bedford M J (1967) Mechanisms of the host in the eye IV The anterior eye in germ free animals *Arch Ophthal (Chica o) ı* 39?–399
R D & Landers D F (1967) Electron and light microscopic observations on relationships between lymphocytes and intestinal epithelium *Amer J Anat 1°1* ı63–ıı4
M S (1960) *Cytology of the conjunctival fluid Acta ophthal (Kbh) Suppl ɔ9*
dnot M (1971) Les proprietes ultrastructurales de la surface de la conjonctive oulbaire *Arch Ophtal (Paris) 31* ı09–716
in J A G (19ı4) *Histology* pp 8 4–898 Oxford University Press New York London Toronto
R M Wittner M & Lenger M (1969) Mitochondrial and other ultra structural changes in great alveolar cells of oxygen adapted and poisoned rats *Lab Invest °0* 516–ɔ°8
arawy M & Penney D P (1973) Unusual mitochondrial morphology in the rat adrenal cortex following hypophysectomy *Amer J Anat 136* 39ɔ–401
H W (1975) Ultrastructure of cell migration through the gastric epithelium and its relationship to bacteria *J clin Path °8* 639–646
tephens R J & Bils R F (1965) An atypical mitochondral form in normal rat liver *J Cell Biol °4* 500–504

Suyama A T Long J A & Ramachandran J (19//) Ultra tructural ch... duced by ACTH in normal adrenocortical cells in culture *J Cell Biol* 2...
Tenner C Racz P & Redey B (1971) Significance of the epithelial pha... e... perimental salmonella conjunctivitis *Acta Microbiol Acad Sci Hun* 18 ...
Weingeist T A (19/3) The conjunctiva *Int Ophthal Clin 13* 85–91
Yates R D (1965) Fine struc ural observations on untreated and ACTH... adrenocortical cells of the zona re iculari of syrian hamsters *Z Zellforsch* ... *Anat 66* 384–395
Zelander T (1959) *Ultrastructure of mouse adrenal cortex J Ultra...* *Suppl* 2
Zucker Franklin D (1969) The ultrastruc ure of lymphocytes *Sem Hemat* ...

Author s address

Dr Stefan Latkovic
Department of Ophthalmology
University of Linkoping
S 581 85 Linkoping
Sweden

Department of Ophthalmology (Head Sven Erik G Nilsson)
University of Linkoping Sweden

THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

IV The Palpebral and the Perimarginal Zones

BY

STEFAN LATKOVIC

The ultrastructure of the palpebral and perimarginal zones is described and compared to that of the other zones of the conjunctival epithelium of the guinea pig The palpebral zone was characterized by frequently occurring goblet cells the structure and function of which are discussed The transition in the perimarginal zone from a four layered epithelium with cylindrical superficial cells in the palpebral zone to a multilayered squamous epithelium towards the lid margin is shown.

Key words conjunctival epithelium – palpebral zone – perimarginal zone – goblet cell – guinea pig – ultrastructure – transmission electron micro scopy

present description of the palpebral and perimarginal zones concludes a ies of papers concerning the ultrastructural survey of the normal conjunctival lium of the guinea pig The preceding papers described the perilimbal (Latkovic & Nilsson 1979a b) the bulbar zone the zone of the fornix and supranodular zone (Latkovic 1979) The aim of the study was also to pro a background for an investigation of phagocytosis in conjunctival epithe cells (Latkovic & Nilsson 1979c)

The palpebral zone comprising the longest part of the conjunctival epithe m begins at the distal limit of the supranodular zone and merges into the arginal zone which in turn constitutes the zone of gradual transition from

ceived July 6 19 8

the conjunctival epithelium to the keratinized epidermis at the lid ma... palpebral zone and to some extent also the perimarginal zone included ... of the basic cell types described in the preceding papers (Latkovic & N... 1979a b) The present paper therefore concentrates on the morphologica... ferences between the zones described here and those reported on earlier...

Previous electron microscopic studies of the palpebral/tarsal conjunctiva concern human material Greiner et al (1977) described the surface morph... of the conjunctiva as seen in scanning electron microscopy Dark et al. ... focused on histochemical and transport problems Both studies concerned superficial cells and were discussed in detail in the paper on the super... layer of the perilimbal zone (Latkovic & Nilsson 1979b) Takakusaki ... described the basic cell types of human tarsal conjunctiva and the cha... vernal conjunctivitis A picture of the tarsal conjunctiva was published ... a paper by Weingeist (1973a) The perimarginal zone was described brief... a survey of vertebrate epithelia (Parakkal & Alexander 1972)

Material and Methods

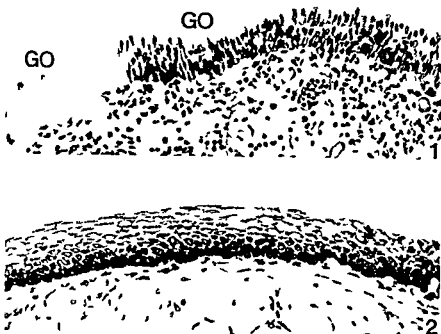
Conjunctival specimens from four clinically healthy pigmented guinea ... were prepared for light and electron microscopy according to the method... scribed in detail in the preceding papers (Latkovic & Nilsson 1979a b)

Observations

The palpebral zone

The palpebral zone was the longest part of the guinea pig conjunctiva, and... general appearance is seen in Fig 1 which is a light micrograph The ep... lium showed a rather uniform configuration with minor variations concer... number of cell layers and incidence of goblet cells There was a rather di... *border towards the epithelium of the supranodular zone* while at the oppo... end the transition to the epithelium of the perimarginal zone was gradual

The ultrastructural characteristics of the epithelium of the palpebral zone ... demonstrated in a survey electron micrograph (Fig 3) The basal l... composed of basal cells and occasional small lymphocytes (Fig 3) Melano... and melanosomes were absent The intermediate layer generally contai... to two layers of polyhedral cells These cell types were similar to the c... responding ones in the perilimbal zone Immature goblet cells at different ... of development were also observed in the intermediate layer (Fig 3) In ... with a small number of mucous globules the latter were distributed rand...



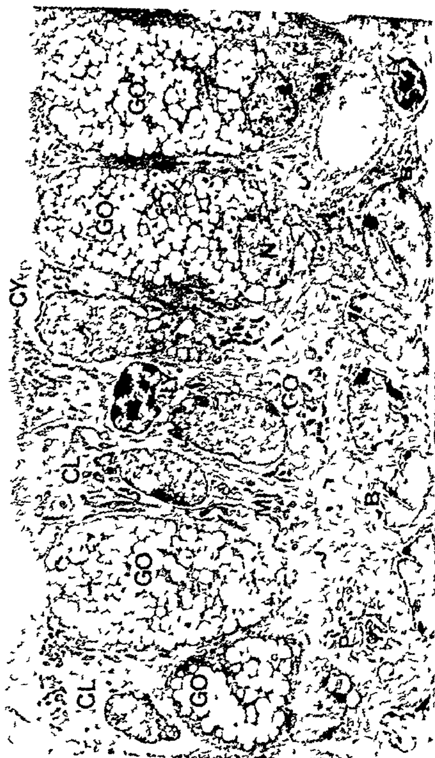
Figs 1 and 2

Survey light micrograph of the conjunctival epithelium of the palpebral zone The l layers number 3–4 Lightly stained goblet cells (GO) in the superficial cell layer seen either solitary interspersed between the cylindrical cells or in rows The junctival stroma contains a large number of cells × 340

Survey light micrograph of the epithelium of the perimarginal zone The transition m cuboidal cells (to the right) to squamous cells (to the left) is demonstrated as is increasing number of cell layers The dark appearance of the cells in the basal and rabasal layers is due to the presence of melanin The nuclei of the cells constituting outer half of the squamous epithelium are very lightly stained The conjunctival na is characterised by a paucity of cellular elements × 350

the cytoplasm while the nucleus was large and located centrally With in ing mucous content the cell assumed an elongated shape with the upper ll compartment packed with mucous globules the nucleus displaced to the of the cell and the amount of cytoplasmic matrix and the number intracellular organelles correspondingly decreased (Fig 3)

The superficial cell layer consisted of cylindrical epithelial cells and mature oblet cells often in groups alternating in an irregular way (Fig 3) Both cell were greatly elongated and sometimes even represented more than half


GO
GO
CY
N
B
GO
CL
CL
B
GO
CL
GO

e epithelial thickness In such a case the intermediate cell layer could be nt (Fig 3) The cylindrical cells did not differ from those of the zone of fornix They contained vesicles vacuoles and intracellular cysts (Fig 3) of same kind as observed in the superficial cells of the perilimbal and bulbar es and the zone of the fornix The free cell surface was covered with micro i Towards the perimarginal zone the shape of the superficial cells was more oidal

he mature goblet cell was almost entirely occupied by tightly packed mucous bules single or coalesced and sometimes seen to be membrane bound (Figs 3 4) Some globules showed a single electron dense core or granule The in paces between the globules were filled with electron dense granulated ma al in which ribosomes and occasional mitochondria could be identified 4) At the free surface the goblet cell was either covered by a thin layer cytoplasm and a cell membrane with microvilli or it could be open (Figs 3 1 5)

he tightly interdigitating cytoplasmic processes and the intercellular junc ns of the cylindrical and goblet cells were similar to those of the superficial s of the previously described zones

mall lymphocytes were the only migrating cells found in the palpebral zone l they were observed in all cell layers (Fig 3)

perimarginal zone

one of transitional epithelium the perimarginal zone is interposed between palpebral conjunctiva and the lid epidermis Adjacent to the palpebral zone led the conjunctival epithelium and at the lid margin it was similar to epidermis The middle part consisted of a multilayered squamous non ing epithelium

A survey electron micrograph (Fig 6) shows the basal and intermediate cell ers of a section from an area not too far from the palpebral zone The basal with basal cells and melanocytes closely resembled the corresponding

Fig 3

v power survey electron micrograph of the conjunctival epithelium of the palpebral The superficial layer consists of cylindrical cells (CL) and rather numerous goblet s (GO) An intracellular cyst (CY) is seen in a superficial cell Immature goblet s are found in the intermediate layer which is rather indistinct in this figure Small cytes (LY) are present in the basal and superficial layers B basal cell P poly hedral cell in the intermediate layer × 3850

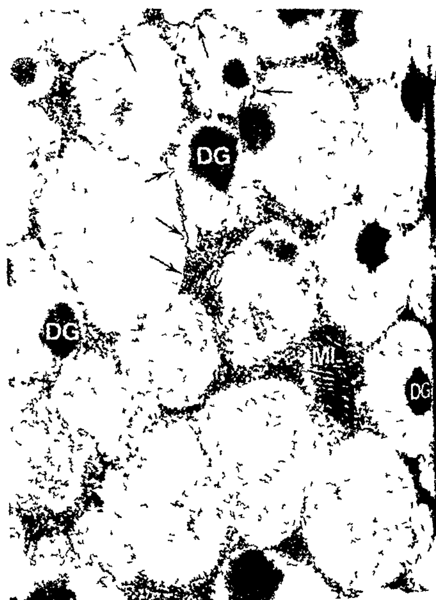


Fig 4

Electron micrograph of mucous globules from a goblet cell Enclosing membra[ne] be observed in certain places (arrows) Some globules appear partly coalesced [...] variation in electron density between different globules is noticed and someti[mes] electron dense granule (DC) is present Mitochondria (MI) and ribosomes are [...] the dense granular material filling the interglobular spaces × 33 000



Fig 5
anning electron micrograph of a group of goblet cells (GO) more or less open at the surface CL cylindrical cells Arrows point to the intercellular borders × 4000

of the perilimbal zone The presence of melanin (Fig 6) caused the dark rance of the cytoplasm of these cells observed also in light micrographs ig 9) The suprabasal and inner intermediate cell layers were composed of ier flat polyhedral cells with a moderate quantity of cytoplasmic organelles



MF
P
DP
P
DP
P
MC
B
B
DP
M
DP

› 6) Transversely cut dendritic processes with a smooth cell membrane and ence of intercellular junctions were observed between the basal and poly lral cells (Fig 6) They could be identified as melanocyte extensions only en containing melanosomes (Fig 6) As the sections were cut farther away m the palpebral zone the cells of the outermost intermediate layers were n to be much flatter (Fig 7) The number of microfilament bundles was de a ed What appeared to be partly degraded mitochondria were observed in ne cells (Fig 7) The cytoplasmic processes were shorter in the outer layers e superficial cells showed a cytoplasm with few microfilaments some vesicles d ribosomes and a free surface covered by microvilli (Fig 7)

In the periphery of the perimarginal zone the epithelium had the configura n of a multilayered nonkeratinizing squamous epithelium (Fig 8) The number cell layers was further increased primarily due to numerous layers of very t squamous cells in the outer part of the epithelium as compared to the area presented by Figs 6 and 7 The nucleus was flat and lightly stained The toplasm contained a moderate number of fibrillar strands some vacuoles d a large number of dense particles (Fig 9) Desmosomes occurred rather quently (Fig 9) The microvilli were absent on the free surface of the super ial cells The inner part of the epithelium consisted of a rather large number polyhedral cell layers (Fig 8) and a single basal cell layer The majority of lei contained minimal quantities of chromatin and appeared less dense than e cytoplasm both in light (Fig 2) and in electron micrographs (Fig 8) Fibril r material was present in somewhat greater amounts than in the cells of the iter layers The mitochondria were few and often appeared as vacuoles with sidues of cristae (not shown) Other cytoplasmic organelles were observed ily rarely

Fig 6

ie basal and deeper intermediate cell layers of the perimarginal zone from a section t too far from the palpebral zone The intermediate cell layers are numerous The olyhedral cells (P) in the deeper intermediate layers appear almost identical in shape cytoplasmic content Dendritic processes (DP) are observed in the basal as well as the intermediate layers When such processes contain melanosomes they can be itified as melanocyte processes (MP) Most of the melanosomes (M) are found in basal layer concentrated in melanocytes (MC) but also to a less extent in basal cells (B) × 6900


S
F
D
N
VE
F
G
VE
F
N
MI

Discussion

꞉e palpebral zone of the guinea pig conjunctival epithelium showed general uformity to the palpebral/tarsal zone of the human conjunctiva as described Takakusaki (1969) Weingeist (1973a) Dark et al (1974) and Greiner et al 77) The main difference was the shape of the superficial cells which was oidal in the human (Takakusaki 1969 Weingeist 1973a Dark et al 1974) ereas in the guinea pig it was mainly cylindrical However at the periphery the palpebral zone the cell shape was more cuboidal even in the guinea pig *thelium Takakusaki (1969) reported an increased number of filaments in* e superficial cells which was not observed in our material

Characteristic for the conjunctival epithelium of the palpebral zone of the ver eyelid was the large incidence of goblet cells particularly in the part jacent to the supranodular zone It should be pointed out that the present vey is based on thin sections cut in the meridians along the middle of the elid and that the distribution of goblet cells along other meridians might be fferent as was demonstrated for human conjunctiva by Kessing (1968) In present material goblet cells were not found in the basal cell layer Imma re goblet cells were first observed in the suprabasal cell layer In studies on e origin of goblet cells in the small intestine Merzel & Leblond (1969) Cairnie 970) and Cheng (1974) concluded that immature mucous cells derive from e transformed columnar epithelial cells and that the number of such im ature mucous (goblet) cells further increased by division

The goblet cells in the conjunctival epithelium of the guinea pig were similar the goblet cells observed in the human conjunctiva (Wanko et al 1964 akakusaki 1969 Radnot 1971 Weingeist 1973b) and throughout the epithelial ning of the respiratory and digestive tracts (Freeman 1966 Cheng 1974 Rho in 1974) Two subgroups of goblet cells one containing mucous globules with ense granules and another without such granules were observed in mouse nall intestine (Cheng 1974) and also in our material The granular and non

Fig 7

superficial (S) and outer intermediate cell layers of the perimarginal zone from section closer to the palpebral zone than to the lid margin The cells are rather flat the cytoplasm is seen to contain a number of larger vesicles (VE) which are con ed in the apical part of the cell and short bundles of microfilaments (F) nucleus MI partly decomposed mitochondrion MV microvilli D desmosomes × 12 900


F
D
F
D

nular mucous globules might possibly represent sequential developmental es

t is generally accepted that the mucus derived from the goblet cells in the junctiva forms a mucous coat on the surface of the corneal and conjunc al epithelium (Wolff 1946 Mishima 1965) Since the critical surface tension mucus exceeds that of tear fluid a continuous tear film is securely held in ce (Lemp et al 1970 Holly & Lemp 1971) Isolated mucous strands/particles the surface of the conjunctival epithelium were shown by Pfister (1975) and Greiner et al (1977) The ultrastructure and enzyme activity of the mucous ead were described by Egeberg & Norn (1967) and Norn (1971) No mucous er was seen in our material Either it was dissolved during the preparation ocedure or it was not demonstrable with the technique used The filamentous ections from the free surface of the superficial cells observed in our mate l which are considered to be different from the mucus and an integral part the cell membrane were discussed in a previous paper (Latkovic & Nilsson 9b)

The transition from the conjunctival epithelium of the palpebral zone to the dermis at the lid margin which could be followed across the perimarginal ne was similar to that described by Larakkal & Alexander (1972) Certain of the epithelium of the perimarginal zone resemble other stratified amous nonkeratinized epithelia such as the buccal and palatal epithelium lverman 1971) and the epithelium of the soft palate and of the oesophagus odin 1974) Towards the lid margin the epithelium began to show certain ns that have been described as preceding keratinization (Brody 1968 Ma tsy 1976) decomposition of nucleus and cytoplasmic organelles increased ount of filaments in the outer layers and absence of microvilli on the epi ial surface

Figs 8 and 9

Survey electron micrograph of the epithelium at the proximity of the lid margin the large number of cell layers The inner layers are composed of cells with lyhedral shape while the outer layers consist of very flat squamous cells The amount nuclear chromatin is minimal and most nuclei (N) are more electron lucent than e cytoplasm x 2200

Electron micrograph of squamous cells in the outer layers of the perimarginal zone ther close to the lid margin Fibrillar material (F) and dense particles are the main mponents of the cytoplasm D desmosomes x 8500

Acknowledgment

This investigation was supported by grants from the Swedish Medical Research C (Project No 12X 134) and the Research Committee of the Östergotlands Lans Le ting

References

Brody I (1968) The epidermis In J Jadassohn Ed *Handbuch der Haut und schlechtskrankheiten Ergan ungswerk I* Springer Verlag Berlin Heidelberg York

Cairnie A B (1970) Renewal of goblet and Paneth cells in the small intest *Tissue Kinet 3* 35–45

Cheng H (1974) Origin differentiation and renewal of the four main epithelial types in the mouse small intestine II Mucous cells *Amer J Anat 141* 481

Dark A J Durrant T E McGinty F & Shortland J R (1974) Tarsal conjunctiva the upper eyelid *Amer J Ophthal 77* 555–564

Egeberg J & Norn M S (1967) Ultrastructure of mucous thread in inferior conjunctival fornix *Acta ophthal (Kbh) 45* 727–732

Freeman J A (1966) Goblet cell fine structure *Anat Rec 154* 121–148

Greiner J V Covington H I & Allansmith M R (1977) Surface morphology of human upper tarsal conjunctiva *Amer J Ophthal 83* 892–905

Holly F J & Lemp M A (1971) Wettability and wetting of corneal epithelium *Exp Eye Res 11* 239–250

Kessing S V (1968) *Mucous gland system of the conjunctiva A quantitative anatomical study* pp 36–50 *Acta ophthal (Kbh) Suppl 95*

Latkovic S (1979) The ultrastructure of the normal conjunctival epithelium of guinea pig III The bulbar zone the zone of the fornix and the supranodular *Acta ophthal (Kbh)* (in press)

Latkovic S & Nilsson S E G (1979a) The ultrastructure of the normal conjunctival epithelium of the guinea pig I The basal and intermediate layers of the perilimbal zone *Acta ophthal (Kbh) 57* 106–122

Latkovic S & Nilsson S E G (1979b) The ultrastructure of the normal conjunctival epithelium of the guinea pig II The superficial layer of the perilimbal zone *ophthal (Kbh) 57* 123–135

Latkovic S & Nilsson S E G (1979c) Phagocytosis of latex microspheres by epithelial cells of the conjunctiva *Acta ophthal (Kbh)* Submitted for publication

Lemp M A Holly F J Iwata S & Dohlman C H (1970) The precorneal tear film I Factors in spreading and maintaining a continuous tear film over the corneal surface *Arch Ophthal (Chicago) 83* 89–94

Matoltsy A G (1976) Keratinization *J invest Derm 67* 20–25

Merzel J & Leblond C P (1969) Origin and renewal of goblet cells in the epithelium of the mouse small intestine *Amer J Anat 124* 281–306

Mishima S (1965) Some physiological aspects of the precorneal tear film *Ophthal (Chicago) 73* 233–241

Norn M S (1971) Enzyme activity in the conjunctival mucous thread *Acta ophthal (Kbh) 49* 528–538

-akkal P F & Alexander N J (19?) *Keratini ation A Survey of Vertebrate Epi helia* pp 34–45 Academic Press New York and London
ster R R (1975) The normal surface of conjunctiva epithelium A scanning electron microscopic study *Invest Ophthal 14* 267–279
dnot M (1971) Beitrag zur Feinstruktur der perilimbalen Zone der Bindehaut *Klin Mbl Augenheilk 159* 421–426
odin J A G (19 4) *Histology* pp 66–89 506–511 Oxford University Press New York London, Toronto
verman S (1971) Nonkeratinization and keratinization the extremes of the human ran e. In Squier C A and Meyer J Eds *Current Concepts of the Histology of Oral Mucosa* C C Thomas Springfield
kakusaki I (1969) Fine structure of the human palpebral conjunctiva with special reference to the pathological changes in vernal conjunctivitis *Arch histol jap 30* ?4?–289
anko T, Lloyd Jr B J & Matthews J (1964) The fine structure of human con junctiva in the perilimbal zone *Invest Ophthal 3* 285–301
eingeist T A (1973a) The conjunctiva *Int Ophthal Clin 13* 85–91
eingeist T A (1973b) The glands of the ocular adnexa *Int Ophthal Clin 13* ?45–?61
olff F (194?) The muco cutaneous junction of the lid margin and the distribution of the tear fluid *Trans ophthal Soc U K 66* ?91–308

uthor s address
Stefan Latkovic
partment of Ophthalmology
niversity of Linkoping
581 85 Linkoping
eden

Hans Sautter Wolfgang Straub & Hermann Rossmann Atlas of the Ocular Photographs of Typical Changes in Ocular and Systemic Disease [... Schwarzenberg Munchen Wien Baltimore 1977 160 pages 37, coloured photographs (with single exceptions) and accompanying text and subject Price D kr 457 75

The book is not only a photographic atlas but also a collection of ultra... histories as each photograph is accompanied by a brief explanatory text ... many cases also contains clinical points of important nature

The many colour photographs of the fundus are grouped into the following ... The fundus of ophthalmological experimental animals the normal human f... physiological variants and congenital malformations diseases of the optic nerve ... seases of the retina diseases of the choroidea and traumatic lesions The present ... comprises a comprehensive photographic material concerning the common ophthalmological diseases Vascular disorders are especially clearly and extensively represented Furthermore several of the more rare ophthalmological disorders have also been included in the book

Each photograph together with the accompanying text represents a clear ... situation The photographic quality and reproduction is of a high standard The book can be highly recommended to ophthalmologists neurologists and internal ... specialists alike

Etlif Gr...

Greve E L (ed) Medical Therapy in Glaucoma W Junk by Publishers The H... 1977 137 pages Price approx 45 Dutch Glds

This is volume 12 in the Documenta Ophthalmologica Proceedings Series

The reported symposium on medical therapy in glaucoma took place in Amsterdam in 1976 The symposium dealt with cholinergic drugs including ocusert pilocarpine and adrenergic drugs and their effect in the treatment of glaucoma with ... mention of the β adrenoreceptor blockings drugs The final chapter of the book ... with the clinical aspects of the medical treatment of glaucoma P Graham (Ca... discusses the very important criteria for initiation of treatment in patients with ... pected glaucoma E L Greve (Amsterdam) discusses some aspects of the management of glaucoma patients Finally Redmond Smith (London) discusses the medical ... the surgical therapy of glaucoma patients In a sensible and well balanced way the small book deals with aspects of the treatment of glaucoma and is valuable to ... not least the practising ophthalmologists who are concerned with the treatment of this often difficult group of patients

S E Lorentzen

Oslo University Eye Department (Head Thore L Thomassen)
Rikshospitalet Oslo

ULTRASONOGRAPHIC STUDY ON CHANGES IN AXIAL EYE DIMENSIONS AFTER ENCIRCLING PROCEDURE IN RETINAL DETACHMENT SURGERY

BY

JON S LARSEN and PER SYRDALEN

The pre and postoperative results of ultrasonographic measurements on the axial ocular components in 10 phakic eyes with retinal detachment treated with encircling silicone rubber band are presented A significant increase ($P < 0.001$) in axial eye length from 0 62 to 1 94 mm (average 0 93 mm) was found The elongation of the eye was caused by a corresponding increase in the length of the vitreous cavity No significant changes were found in the anterior segment of the eyes These data demonstrate that the postoperative refractive change in a myopic direction which an encircling procedure with moderate indentation often produces is caused by an axial elongation of the eye

Key words ultrasonography – retinal detachment – encircling procedure – axial eye length

trasonographic measurements on eyes with retinal detachment treated with an ircling procedure have shown that this procedure often changes the axial igth A shortening seems to be as usual as an elongation (Miettinen & Ursin 68 Flament 1973 Burton et al 1977) A myopic shift in refractive power in our experience often produced by a moderate indenting equatorial en cling procedure We have not observed changes in the parameters of the terior segment The myopic change has therefore been thought to be pro ced by an increase in axial length

ceived January 11 1979

The purpose of the present study was to demonstrate possible ch... ocular parameters (corneal radius of curvature anterior chamber dep... thickness vitreous length and axial length) induced by this method of e... ling procedure This could clarify the cause of a postoperative change ... fraction

Material and Methods

The material includes 10 patients (6 males 4 females average age ...yr (14–76)) with retinal detachment in 10 phakic eyes All eyes were treated ... an encircling silicone rubber band of 2 mm width (Labtician Com... S 2969) combined with small silicone rubber explants under the band ... to create the local buckling In all cases cryopexy and drainage of s... fluid were performed The band was tightened appropriately to ... moderate equatorial indentation

The corneal radius of curvature was determined with the Javal Schiotz ke... ratometer (Haag Streit) in the main meridianes preoperatively and ... postoperatively The anterior chamber depth (including corneal thickness) l... thickness and vitreous length were measured ultrasonographically pre... tively and repeated 5 to 8 weeks postoperatively An A scan echograph ... technic model 7200 MA and a 10 MHz/5 mm transducer (NM 10 ...) ... used The measuring technique is described in detail in a previous ... (*Larsen 1979*)

All measurements were performed under cycloplegia An average of ... measurements was obtained pre and postoperatively of each eye The ... ocular distances were expressed in millimeters using the following ultra... velocities 1532 m/sec in the anterior chamber and vitreous and 1641 m/... in the lens (Jansson & Kock 1962)

The reliability of the method was tested on one eye with retinal de... ment Five measurements were carried out in each of two separate se... (A B) and the data are listed in Table I The maximal deviations from ... values based on these 10 measurements were anterior chamber dep... ... mm lens thickness 0 08 mm vitreous cavity length 0 18 mm and axial length 0 17 mm *Any difference between pre and postoperative values la...* than these maximal deviations are in the following considered as rea...

In order to obtain the total length of the optical system (axial len... thickness of the retina 0 4 mm was added to the calculated axial length (François & Goes 1971)

Refractive power could be determined preoperatively in seven eyes ... postoperative refractive changes are based on these cases

Table I

Axial dimensions in the control eye measured in two separate sessions (A B)
Mean values of 5 measurements each session

Axial eye dimensions	Series of measurements			
	A		B	
	Mean mm	SD	Mean mm	SD
Anterior chamber depth	3 61	0 05	3 62	0 03
Lens thickness	4 46	0 03	4 48	0 06
Vitreous length	18 83	0 11	18 81	0 11
Eye length	26 90	0 11	26 91	0 15

Results

able II shows the pre and postoperative mean values of the minimum and aximum meridional corneal powers As evident from this table no impor int differences were found either between the average minimum (0 04 dptr) r between the maximum meridional corneal powers (0 20 dptr) generated by te encircling procedure The postoperative power changes in spherical equi alents were between –0 5 dptr and +0 5 dptr in eight eyes and between 0 5 dptr and –1 25 dptr in two eyes

Table III shows the pre and postoperative data on the axial eye length The ncircling procedure produced a significant elongation of the axial length in ll eyes examined The average elongation was 0 98 mm (± 0 20 mm) with

Table II

Pre and postoperative corneal curvature in diopters

Major meridian power	Preoperative corneal curvature dptr		Postoperative corneal curvature dptr	
	Mean	SD	Mean	SD
Minimum	42 09	0 79	42 13	0 79
Maximum	42 93	0 75	43 13	1 07

Table III

Axial length in 10 phakic eyes before and 5 to 8 weeks after encircling procedure

Patient No	Preoperative axial length (mm)	Postoperative axial length (mm)	Postoperative lengthening
1	23 22	24 27	1 05
2	23 40	24 02	0 62
3	23 53	24 [illegible]5	1 2
4	23 67	24 91	1 24
5	23 71	24 69	0 98
6	24 11	25 06	0 95
7	24 17	25 00	0 83
8	24 69	25 58	0 89
9	24 84	25 73	0 89
10	25 42	26 61	1 19
Mean	24 08	25 06	0 98
SD	0 71	0 75	0 20

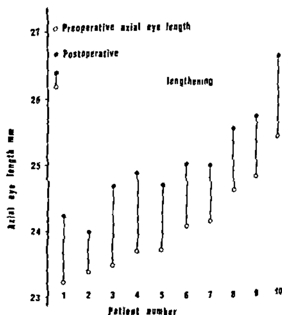


Fig 1

Increase in axial eye length produced by encircling operation

Table IV

Anterior chamber depth and lens thickness before and 5 to 8 weeks after encircling procedure

Patient No	Anterior chamber depth mm		Lens thickness mm	
	Preop	Postop	Preop	Postop
1	3 92	3 91	3 58	3.59
2	3 42	3 41	4 66	4 67
3	3 54	3 53	4 15	4 13
4	3 63	3 62	4 48	4 47
5	3 11	3 07	4 86	4 86
6	3 73	3 71	4 35	4 36
	3 82	3 82	3 69	3 67
8	3 06	3 01	4 66	4 67
9	3 90	3 88	3 84	3 86
10	3 78	3 77	3 81	3 82
Mean	3 59	3 57	4 21	4 21
SD	0 31	0 32	0 46	0 46

ktreme values between 0 62 mm and 1 24 mm (Fig 1) The results showed at all eyes had a corresponding significant elongation of the vitreous cavity he average increase of vitreous length was 1 00 mm (± 0 20 mm) with ex me values between 0 62 mm and 1 26 mm There was a positive correla on between the postoperative elongation of the axial lengths and the cor nding data for the vitreous lengths the correlation coefficient being = 0 997 $P < 0\ 001$

The pre and postoperative data on the anterior chamber depth (included ickness of cornea) as well as on lens thickness are shown in Table IV No sential differences in any of the individual measurements were found for ese parameters The average preoperative anterior chamber depth was 3 59 im (± 0 39 mm) compared to 3 57 (± 0 32 mm) in the postoperative measure ients The average lens thickness was 4 21 mm (± 0 46 mm) in the pre and ostoperative measurements The mean difference between the pre and post perative refraction power was -2 4 dptr (± 1 31 dptr)

Discussion

Experimental encircling procedures with silicone bands of 2 mm w[illegible] formed on eyebank eyes (IOP 20 mmHg) have shown that the [illegible] axial length depends on the tightening of the band (Rubin 19[illegible]) [illegible] to his study a low moderate and high indentation of the band (corres[illegible] to a mean reduction in outer equatorial diameter of 0 42 mm 0 93 m[illegible] 2 1 mm) produced the following changes in the mean axial length 0 [illegible] +1 09 mm and –0 35 mm The average axial elongation of 0 98 mm [illegible] our study corresponds approximately to the elongation in axial len[illegible] 1 09 mm produced by a moderate band indentation obtained in Rubin's studies These facts indicate that an encircling procedure with moder[illegible] dentation of the band will produce an elongation of the eye, thus [illegible] an essential refractive change

The disparity in results found in studies on this topic (Miettinen & [illegible] 1968 Flament 1973 Burton et al 1977) is possibly caused by differ[illegible] gical techniques and tightening of the encircling band The surgical pro[illegible] must also have an influence on the axial dimensions of the anterior [illegible] ment Contrary to our findings which did not show any changes in [illegible] terior segment dimensions (Table IV) other authors have found shallow[illegible] of the anterior chamber (Fiore & Newton 1970 Hartley & Marsh 19[illegible] ton et al 1977) and increased lens thickness (Burton et al 1977) after [illegible] ling procedures Some authors concluded that the shallowing of the [illegible] chamber was transitory or possibly transitory (Fiore & Newton 1970 [illegible] study included no cases with a postoperative choroidal detachment

The refractive power of any eye depends both on the axial length and the refractive power of the ocular components We could not show [illegible] changes in the anterior segment dimensions (Table IV) or in the cor[illegible] fractive power (Table II) The myopia which regularly occurs must [illegible] fore be caused by the axial elongation According to Rubin (1975) 1 [illegible] axial increase produces 2 564 dptr of myopic shift at the spectacle lens [illegible] in the phakic eye (the Gullstrand schematic eye) If this factor is used for eyes in our investigation the approximate values for the postoperative [illegible] tive changes can be estimated According to this the encircling proced[illegible] to changes in a myopic direction from –1 59 dptr (0 62 mm axial el[illegible] to –3 18 dptr (1 24 mm axial elongation) the average refractive cha[illegible] –2 51 dptr (0 98 mm axial elongation) This corresponds in a hi[illegible] to the mean refractive change of –2 4 dptr found in our study Myopic [illegible] of this magnitude have also been shown by Jacklin (1971) and Rubin (1[illegible] The present study has shown that the increase in axial length occur[illegible] larly This fact has to be considered when the operative procedure for [illegible] tinal detachment is planned

References

ton T C Herron B E & Ossoinig K C (197/) Axial length changes after retinal etachment surgery *Amer J Ophthal 83* 59–62

re J V Jr & Newton J C (19/0) Anterior segment changes following the scleral uckling procedure *Arch Ophthal (Chicago) 84* 284–28/

ment J (1973) Étude optico echographique des modifications biometriques de l axe ntero posterieur de l œil provoquees par quelques techniques de la chirurgie chorio etinienne *Arch Ophtal (Paris) 33* 39 –412

nçois J & Goes F (1971) Echographic study of the lens thickness as a function of he axial eye length in emmetropic eyes of the same age In Bock J and Ossoinig K ds *Ultrasonographia Medica* pp 531–538 Wiener Med Akademie Vienna

rtley R E & Marsh R J (1973) Anterior chamber depth changes after retinal de achment *Brit J Ophthal 57* 546–550

klin H N (1971) Refraction changes after surgical treatment of retinal detachment *th med J 64* 148–150

F & Kock E (1969) Determination of the velocity of ultrasound in the human ens and vitreous *Acta ophthal (Kbh) 40* 420–433

rsen J S (19/9) Axial length of the emmetropic eye and its relation to the head ize *Acta ophthal (Kbh)* (in press)

ettinen P & af Ursin K V (1968) Ultrasound studies on changes in the length of erclage operated eyes In Vanycek J Ed *Symposium internationale de diagnostica ultrasonica in ophthalmologia* pp 939–943 Universita J E Purkyne Brno Czecho slovakia

bin M L (1975) The induction of refractive errors by retinal detachment surgery *Trans amer ophthal Soc 73* 459–490

thor s address

r Syrdalen M D
niversity Eye Department
kshospitalet
slo 1
orway

Department of Ophthalmology Karolinska Hospital Stockh...
(Head B Tengroth)
and Department of Ophthalmology University Hospital Upp...
(Head L. Berggren) Sweden

RETINOBLASTOMA IN SWEDEN 1958–1971

A Clinical and Histopathological Study

BY

ERIK KOCK and PETER NAESER

This study includes all cases of retinoblastoma reported in Sweden betwe... 1958 and 19 1 The incidence of the disease was 1 per 15 000 live b... Only in six cases was there a familial history and five of thes cases w... bilateral. The tumour was bilateral in 3 ... of all cases All cases w... unilateral tumour had been treated with enucleation. In the bila eral cas... one eye had also been enucleated and the other eye trea ed by local ... diation therapy Tumour invasion into choroid was found in 29... in... into the optic nerve in 11 % of the cases. The mortality was only 15...

Key words Retinoblastoma – clinic – histology

Retinoblastoma is the most common intraocular tumour in childhood. D... the past years it has often been discussed whether there has been an ... in the incidence or manifestation of this malignant disease (Ta... Tuovinen 1971 Bishop & Madson 1975) It was therefore decided to ... clinical and histological aspects of all cases of retinoblastoma reported in S... over a 13-year period and to compare the results with those fr... countries

Received September 2. 19 ..

Material and Methods

ıe present material was obtained from the Swedish Cancer Registry (SCR) ıd comprised 94 patients The data on six of the cases could not be traced in e clinics which had reported them to SCR or from the histopathological re stration number of the cases In the remaining 88 cases the medical records ›tained from 28 different clinicse were studied Histological sections were ›tained from 75 of the cases from 12 different pathological laboratories The ıcroscopic slides had been stained with either haematoxylin and eosin or van ıeson stain Each section was studied by both authors independently with .spect to 19 different variables The mean value of each variable was expres d in per cent of the total number of cases studied

Results

linical observations

he present material comprised 48 boys and 40 girls with retinoblastoma giving n incidence of 1 per 18 000 live births The initial sign was a pupillary change ı 51 cases Squint was noted in 17 cases and acute red eye in 8 cases Of the maining 12 cases three were diagnosed on routine examination because of a amilial history four on clinical examination after head injury and four on ɔutine clinical examination at child health centres In only one case was in ormation lacking about the presenting sign

The duration of the symptoms before diagnosis can be seen in Table I Ap roximately 50 % displayed eye symptoms for less than 2 months In one case glittering pupil was noted by the parents for 2½ years

Table I
Duration of eye symptoms before tumour diagnosis

Duration of symptoms (months)	No of cases
< 1	27
1 – 2	13
2 – 3	11
3 – 6	13
6 – 12	9
>12	6

Table II

Age of the 88 children at the time of diagnosis.

Age	No of cases	
	Total	Bilateral
0 – 3 months	3	[illegible]
3 – 6 months	12	9
6 – 9 months	11	8
9 –12 months	3	[illegible]
12 –18 months	14	[illegible]
18 –24 months	13	4
2 – 3 years	16	3
3 – 4 years	10	0
4 – 5 years	2	0
5 – 6 years	1	0
> 6 years (6 6½ and 9½ years)	3	0

There were 33 bilateral (18 boys and 15 girls) 31 right sided ([illegible] 16 girls) and 24 left sided (14 boys and 10 girls) cases Twenty six of the [illegible]lateral cases were diagnosed at the first examination During the first six [illegible] following unilateral diagnosis a tumour was observed in the second eye [illegible] two cases and during the next six months three more cases were found [illegible] in two cases did a tumour occur in the second eye more than 1? months [illegible]

The age distribution of the children at the time of diagnosis can be seen [illegible] Table II Two thirds of the cases were diagnosed within the first two years [illegible] life After the age of four years only six cases were diagnosed The majority [illegible] the bilateral cases (64 %) were discovered during the first year of life and [illegible] such case was found after the age of three years

A familial history of retinoblastoma recorded in only six (five bila[illegible] cases The clinical records gave no information about familial history in [illegible] (three bilateral) cases

Four patients died from the disease The tumour was located in the right [illegible] in one the left eye in one and bilaterally in two patients In none of the [illegible] patients was there a familial history In two of these cases the initial s[illegible] a pupillary change Both of these cases showed brain metastases 1½ years [illegible] In the other two fatal cases there was an increased intraocular pressure [illegible] acute red eye as a presenting sign In these cases tumour recurred in the [illegible]

e months after the diagnosis and both patients died from the disease within months of the tumour diagnosis

All cases with unilateral tumour were enucleated at the time of diagnosis In bilateral cases enucleation usually had been performed in the eye harbouring largest tumour and the other eye was treated by local irradiation therapy 19 cases the second eye was enucleated either because the tumour primarily considered to be to large for radiotherapy or because changes secondary to therapy made it impossible to inspect the retina

The time of observation varied between six and 19 years after diagnosis

tological observations

ble III gives the results of the different histological variables It can be seen it almost every tumour showed necroses and that calcifications were frequent may also be noted that 29 % of the eyes displayed tumour in the choroid and % showed tumour at or beyond the lamina cribrosa In most cases the tumour med to fill more than half of the sectioned area The macroscopic descrip ns however did not give any exact measure of the tumour size

Table III
Histological findings in the 75 retinoblastoma eyes

Tumour necrosis	97 %
Tumour calcifications	72 %
Perivascular collars	81 %
Endophytic growth	0 %
Exophytic growth	41 %
Multifocal growth	27 %
Diffus retinal growth	4 %
Fleurettes	0 %
Rosettes	50 %
No rosettes	50 %
Mitoses	76 %
Choroidal invasion	29 %
Optic nerve invasion	11 %
Subarachnoidal invasion	1 %
Extra bulbar tumour extension	3 %
Tumour cells in anterior chamber	11 %
Retinal detachment	61 %
Inflammatory reaction in tumour	60 %
Inflammatory reaction in uvea	13 %

Only from three of the four fatal cases were eyes available for exa-(3 eyes) These showed no rosettes and mitoses were prominent. Two showed tumour cells in the choroid The optic nerve could be inspe- in two cases and none showed tumour cells

Discussion

Retinoblastoma has previously been studied by several Scandinavian a- (Horven 1926 Mork 1961 Jensen 1965 Nielsen & Goldschmidt 19- Ta-kanen & Tuovinen 1971 Hörven 1974 Jerndal et al 1973) The frequen- the disease varies In Finland between 1912 and 1919 the frequency w- 1: 82 000 live births (Tarkkanen & Tuovinen 1971) In a small Norwegian m-rial from 1969 to 1971 comprising 16 cases a frequency of 1 per 1-) reported (Horven 1974) However the figure 1 per 18 000 in the prese- terial is similar to that in Denmark (Jensen 1965) and also to that f- other West European countries (cf Bishop & Madson 1975)

The sex and age incidence do not significantly differ from those of countries The side location of the tumour is similar to that reported fro- other Scandinavian countries

A familial history was rare in our material although there is some u- tainty since this investigation was limited to a perusal of the clinical re- However it may be noted that a familial history was rare in earlier Sw- reports (Jereb et al 1967 Jerndal et al 1973) It should however be ex- pected that a large percentage of the bilateral cases were hereditary (Sch- pert Kimmijser et al 1966) It is noteworthy that among the six cases w- a familial history of retinoblastoma there were five boys with bilateral tu- The sixth case was a girl with a right sided tumour

The mortality was very low and only one of the four fatal cases was b- lateral This contrasts with the higher mortality reported previously from other Scandinavian countries where the deaths were 30 % in Finland, 3- in Denmark and 21 % in Norway It is difficult to explain the reason f- considerable difference One possibility is that the therapy of bilateral ca- has improved with the introduction of the local irradiation therapy (S- 1952 Rosengren & Tengroth 1963) Neither the low mortality in the u- ral cases nor the duration of symptoms before diagnosis or the primary tr- ment differ significantly from those of other countries

The histological findings did not significantly differ from other rep- The choroidal invasion in retinoblastoma has been claimed to cons-

sen the prognosis of the disease (Carbajal 1958 1959 Merriam 1950) It owever of interest to note that 29 % of our cases showed choroidal tumour asion In our material this variable did not seem to influence significantly prognosis This agrees with the conclusions of Redler & Ellsworth (1973) presence of the tumour cells in the anterior chamber or infiltration of the our into the optic nerve has not proved to be of prognostic importance in material Although tumour calcifications were very frequent in our mate (79 %) in no case was a radiograph taken

he present study has shown that there is no significant difference in ical or pathological manifestations of the retinoblastoma diagnosed in den compared with those in other Scandinavian countries The mortality vever was lower probably as a result of improved radiation therapy Al ugh the rate of inheritance is low in the present material the findings vever are in accordance with the suggestion that most of the cases with inoblastoma are sporadic (Duke Elder 1967)

References

hop J O & Madson E C (1975) Retinoblastoma Review of the current status *urvey Ophthal 19* 342–366

bajal U M (1958) Observations on retinoblastoma *Amer J Ophthal 45* 391–407

bajal U M (1959) Metastasis in retinoblastoma *Amer J Ophthal 48* 47–69

ke Elder S (1967) *System of Ophthalmology Vol X* p 678

nen E (1926) *Om forekomsten og prognosen av glioma retinae* Norsk Oftalmolo isk Forening Oslo Nov 25 (1926)

rven I (1974) Retinoblastoma in Norway *Acta ophthal (Kbh) Suppl 123* 103–109

sen O A (1965) Retinoblastoma in Denmark 1943–1958 *Acta ophthal (Kbh) 43* 91 340

eb B Kock E & Åsard P E (1967) Prognosis of retinoblastoma treated at Ra iumhemmet 1926–1963 *Acta radiol (Stockh) 6* 369–377

ndal T Lindstedt E Svensson T & Åkerskog G (1973) Retinoblastoma in Sweden A study of 45 children with retinoblastoma with special regard to the therapeutical sults *Acta ophthal (Kbh) 51* 543–550

erriam G R Jr (1950) Retinoblastoma analysis of seventeen autopsies *Arch Ophthal (Chicago) 44* 71–108

ork T (1961) Malignant neoplasms of the eye in Norway *Acta ophthal (Kbh) 39* 874–831

n M & Goldschmidt E (1968) Retinoblastoma among offspring of adult sur vivors in Denmark *Acta ophthal (Kbh) 46* 731–741

dler L D & Ellsworth R M (19 3) Prognostic importance of choroidal invasion in retinoblastoma *Arch Ophthal (Chicago) 90* 294–296

osengren B H O & Tengroth B (1963) A modified Cobalt 60 applicator for the treatment of retinoblastoma *Acta radiol (Stockh) 1* 305–313

pearance function and electroretinogram a follow up period of at lea years to rule out delayed onset in the unaffected eye and exclusion of i flammatory cause in the affected eye (especially syphilis or viral disease) have suggested further strictures such as the presence of a normal EOG fellow eye (Henkes 1966) Refinements in the recording and evaluation troretinograms developed since these criteria were suggested (for example luation of implicit times (Berson et al 1968)) have allowed earlier diag disease in eyes which might previously have been considered normal

The case herein described has the clinical appearance of unilateral re pigmentosa the serologic criteria for luetic retinopathy but the electro logic findings of bilateral retinitis pigmentosa presenting in a most as tric manner

Case Report

I M C a 51 year old Negro female was first seen in August 1974 with a his poor vision OD for an unknown length of time which had recently become much worse" There was no personal history of trauma infection diabetes or tension and no family history of visual problems Uncorrected visual acuity (20 20 Best corrected acuity OD (–0 50 combined with a +1 50 axis 100) was Pupils were felt to be normally reactive Abnormal findings were limited to the of the right eye and consisted of pigment clumping with spicules both in the per and posterior pole markedly attenuated vessels and a pale disc The differential d nosis was luetic retinopathy vs unilateral retinitis pigmentosa A retinal consul the patient and diagnosed luetic retinopathy OD

An FTA ABS was drawn and was positive (VDRL was non reactive) The pat ceived standard therapy consisting of 3 million units Benzathine Penicillin intra larly given at weekly intervals for at total of three doses (9 000 000 units) The no evidence of a Herxheimer reaction Follow up ophthalmologic examina months later was unchanged

The patient returned in January 1976 complaining of further decrease in visual Best corrected V_A at this time was 20/80 +1 OD 20/20 –2 OS Fundus findings essentially unchanged although some vascular sheathing OD was noted by the ex ner The patient was presented to the same retinal consultant who stated she edly had luetic retinopathy OD

In December 1976 acuity had dropped to hand movements OD remaining at –2 OS At this time a right exotropia and amaurotic pupil OD were noted Fu findings were unchanged and a different retinal consultant concurred in the di of luetic retinopathy OD normal fundus OS

In August 1977 acuity OD had dropped to light perception with some proje Acuity remained 20 20 –2 OS with no funduscopic abnormalities noted An ERG obtained which revealed no recordable responses OD and normal amplitude ph and scotopic responses OS (latencies were not determined) EOG ratios were 1 and 1 86 OS (normal greater than 1 50) Fundus photos were obtained OD but the patient refused fundus photography OS



Fig 1
ndus photo OD showing marked pigmentary retinopathy pale disc and attenuated vessels with sheathing

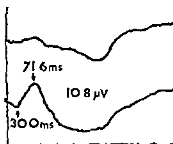


Fig 2
Lower lid skin electrode ERG OU (OD above OS below) 128 responses averaged See Discussion for normal voltage and implicit time values

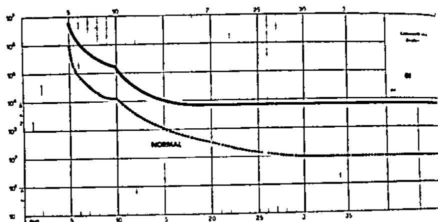


Fig 3

Dark adaptometry OS showing elevated thresholds Normal adaptation [illegible] patient s age included for reference

We were able to examine the patient in April 1978 Best corrected [illegible] OS was 20/25 Fundus examination was unchanged OU The vitreous [illegible] in both eyes Goldmann visual fields showed only a temporal island OD and [illegible] constriction to several isopters OS Standard ERG testing (Cambridge ERG [illegible] using Burian Allen corneal electrodes) revealed non recordable responses OD [illegible] normal voltages OS Scotopic amplitudes OS were reduced by 50 to [illegible] parison with the ERG obtained under identical testing conditions (Cambrid[illegible] protocol) in August 1977 Computer averaged skin electrode ERG testing OS [illegible] prolongation of implicit times (Fig 2) EOG ratios were 1 90 OD 1.55 OS Gold[illegible] Weekers dark adaptometry OS revealed final thresholds to be elevated nearly [illegible] units (Fig 3) Fundus photography OS was again refused by the patient The diag[illegible] was felt to be either markedly asymmetrical retinitis pigmentosa with an [illegible] positive serology or a mild form of tapetoretinal degeneration (RP) with super[illegible] unilateral luetic retinopathy

Discussion

Syphilis may produce a retinopathy whose appearance is clinically [illegible] guishable from retinitis pigmentosa Although acquired luetic choriore[illegible] neuroretinitis is generally a binocular disease the clinical presentation [illegible] unilateral Most commonly the disease is associated with vitreous ha[illegible] retinal vasculitis particularly affecting the arterial side of the circulat[illegible] dence of iritis or focal choroiditis is common Progression does not [illegible] occur after adequate anti syphilitic therapy and final visual acuity is [illegible] fairly good (Falls 1966) While a pseudo retinitis pigmentosa appear[illegible] be present in the fundus discrete chorioretinal lesions are usually [illegible] amid the pigmentary clumps

he diagnosis of luetic retinopathy requires serologic studies often including FTA ABS As Smith (1969) has emphasized the VDRL and even the TPI may be negative in persons with ocular syphilis It is quite likely that some ier case reports of unilateral retinitis pigmentosa were actually cases of negative (by the less specific tests then available) luetic retinopathy

" iss & Nicholl (1968) reported two cases of unilateral retinitis pig itosa in patients with negative FTA ABS and other serologic tests but these hors based their diagnosis on a compatible fundus appearance in one eye normal funduscopy acuity and fields in the other No electrophysiologic dies were performed nor were other possible etiologies (eg trauma vascular ufficiency) ruled out

While syphilis the great imitator may clinically mimic nearly any other ase the presence of a positive serology does not necessarily mean that the angement in question is luetic in origin Serologic evidence of prior syphilitic ection should be considered a necessary although not a sufficient condition the attribution of a luetic etiology to the pathology under consideration

Complete abolition of any recordable electroretinogram would be unusua' though not unknown (Smith 1969)) in luetic retinopathy as would continued ogre sion of disease after adequate treatment In luetic retinopathy as well in other forms of inflammatory retinopathy ERG a and b wave latencies ould remain normal Prolongation of implicit times appears to be a feature of inherited tapetoretinal degenerations

Characteristic of the electroretinogram in tapetoretinal degenerations (retinitis mentosa) are changes in the implicit times of the evoked potentials as well in their amplitudes (Berson et al 1968) While amplitude reduction is pro rtional to the amount of non functional retina in inflammatory vascular aumatic and toxic affections the time course of the residual ERG response unremarkable The tapetoretinal degenerations on the other hand show pro ngation of the temporal course of electrophysiologic responses to light Am itude reductions vary with most forms of retinitis pigmentosa showing early id profound abnormalities while some incomplete forms show relatively mild ductions in amplitude Pruett & Schwarz (1978) have recently described three ses of occult retinitis pigmentosa where visual symptomatology did not be n until the seventh decade of life

We have previously shown (Skalka in press) that photopic computer aver ed skin electrode ERG responses with the Grass photostimulator lamp at S16 tensity and placed four feet from the patient s eye show a wave implicit mes of 23 78 ± 3 68 (± 1 SD) msec and b wave implicit times of 49 92 ± 4 17 ± 1 SD) msec As seen in Fig 2 our patient s a wave implicit time OS of 30 0 sec and b wave implicit time of 71 6 msec show a wave prolongation of nearly

The Department of Ophthalmology (Head Jon O Ihlen) Telemark Sentralsjukehus Skien Norway

INTRAMUSCULAR IRON THERAPY AND TAPETORETINAL DEGENERATION

A case report

BY

KJELL SYVERSEN

A 63 years old male with pernicious anemia had been treated for [illegible] years with weekly injections of iron dextran and cyanocobalamine Oph[illegible] mological examination revealed the ophthalmoscopic picture of a tape[illegible] toretinal degeneration reduced visual acuity and narrow visual fields ERG and dark adaptation test were normal Hematological exam[illegible] including liver and bone marrow biopsies gave no support for the existence of systemic siderosis It is proposed that the retinal degeneration is [illegible] to the extensive parenteral iron treatment with a total dose of appro[illegible] mately 100 grams of iron. This theory is supported by a previous experi mental report

Key words Retinitis pigmentosa – tapetoretinal degeneration – pernicious anemia – parenteral iron treatment – systemic siderosis

In an experimental work Cibis et al (1957) described defective vision [illegible] retinitis pigmentosa like retinal disease in dogs 4 to 7 years after [illegible] injections of iron oxide or multiple blood transfusions The dogs showed [illegible] signs of hepatic cirrhosis or findings similiar to hemochromatosis All the [illegible] who had been kept alive for 7 years after the iron treatment had severe [illegible] nerations in their retinas but only small amounts of iron deposits.

In the same work Cibis et al reported the findings of siderotic [illegible] the retina the pigment epithelium choriocapillaris and the ciliary [illegible]

Received June 23 1978

ent who had been treated with multiple blood transfusions I have not ı able to find any report of eye damage in man caused by parenteral iron apy or hemochromatosis

Case Report

nale farmer born 1914 from unrelated parents and with no family history of tis pigmentosa or other eye diseases In 194 gastric resection was performed ıuse of a peptic ulcer A pernicious anemia was diagnosed in 1957 and since then ıas been treated with cyanocobalaminpreparations in adequate doses Additionally eceived weekly intramuscular injections of iron dextran ([R] Imferon) each injection l containing 50 mg Fe/ml As this treatment was given uninterruptedly for ?0 years total iron dose has been approximately 100 grams

He had no visual impairment until 1973 when he first complained of reduced vision l poor night vision At the time of his first visit to our department in May 1974 general health was good Blood pressure 150/90 Our standard hematological tests luding hemoglobin, serum iron and serum Vitamin B_1 were normal There were ı brown dust like materials on the back surfaces of both corneas and a moderate mentation of the trabecular meshworks Both lenses had pseudoexfoliation of the erior capsules but no cataract or discolouration Ophthalmoscopy and fundus photo phy was to some extent difficult due to a diffuse obstruction to the light trans sion in the vitreous bodies No distinct opacities could be observed Nevertheless was possible to observe the optic discs which were both of normal colour with ysiologic cupping The retinal arteries were slightly narrowed artery/vein rela n = 1/ In the equatorial regions the retinal epithelium showed marked atrophy in iltiple well demarcated patches and dark brown pigment deposits with bone spicula mation

Visual acuity was R E sph –1 cyl 90 –1 5/6 L E sph –0 5 cyl 90?–1 5/8 5 Visual field amination with Goldmann perimeter and Hamblin perimeter revealed narrowing of fields to 10–15° in both eyes Colour vision was normal tested with Ishihara s eudoisochromatic charts and Farnsworth 15 hue test

ERG and dark adaptation tests were not available in the department and the disease as primarily considered to be an idiopatic retinitis pigmentosa Little attention as paid to the hematological aspects as the anemia was kept under control and eated by his general practitioner

During the years 1974–1976 there were no visible changes in the retinal pictures it a gradual loss of vision and peripheral visual fields In Dec 1976 the corrected A was R E 5/1? L E 5/ 0 Up to that time the treatment with cyanocobalamin nd intramuscular iron dextran had ben continued In January 1977 a blood examina on revealed a slight elevation of the E S R (?4 mm/h) Hemoglobin hematocrit rum iron bilirubin alk phosphatase ASAT ALAT and serum Vitamine B_1 were l The ironbinding capacity was slightly decreased (4? mg/l) The treatment with yanocobalamin was kept unchanged but iron dextran was discontinued from Jan 977 Examination in the dept of internal medicine in March 1977 confirmed the iagnosis of pernicious anemia the hematological values showed no changes from hose mentioned above Needle biopsy from the liver showed fatty degeneration and

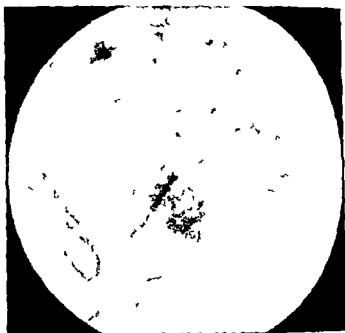


Fig 1
Fundus photograph from right fundus

a special staining for iron revealed some degree of iron pigment depo[illegible] siderosis Liver scintigraphy was normal The conclusion from the medical [illegible] that the patient had a well balanced pernicious anemia and no sign of p[illegible] iron storing

Since March 1977 there has been no progression of the visual loss and in [illegible] of the last five examinations up to Dec 1978 an improvement of the visual [illegible] the visual fields have been noted At the last examination, the V.A. was R.E. [illegible] L.E. 5/6 A reduction in the pigmentations or the atrophic changes in the [illegible] not ben observed ERG and dark adaptation test in June 1978 (Dept of O[illegible] mology Bergen University) were normal A drop in the hemoglobin in [illegible] has responded well to per oral iron medication

Discussion

The coexistence of pernicious anemia and tapetoretinal degenera[illegible] independent diseases cannot be excluded but it is not inconceivable [illegible] might exist a connection between them though I have not been a[illegible] a report of any such coexistence or retinotoxic effect of cyan[illegible] Parenteral iron therapy is usually recommended for a limited period [illegible]

in order to fill the iron depots of the body The effect of more prolonged tment is not fully known

n our case the patient received a total dose of approximately 100 grams nterally The body has a very limited capacity to excrete iron and thus accumulation must have been considerable There is however no sign of iologic storing of iron in the liver or bone marrow

he tapetoretinal degeneration in spite of no pathologic iron storing cor ionds well to the clinical picture in dogs demonstrated by Cibis et al (1957) indicates a retinotoxic effect of prolonged treatment with parenteral iron k of progression and even slight improvement during the time after dis tinuation of the iron therapy may support a causative relationship ERG dark adaptation test were made at a stage of clinical improvement but the mal findings speak against retinitis pigmentosa Our patient received an remely high total dose of parenteral iron and similar cases will probably be and far between

he possibility of eye complications should be born in mind when giving longed treatment with iron injections or multiple blood transfusions

References

il A Cibis Elmer B Brown and Syng Min Hong (1957) Ocular effects of systemic iderosis *Am J Ophthal 44* 158–172

th r s address
ll Syversen
dept
.anien Hospital
)0 Skien, Norway

Department of Ophthalmology (Head E Westerlund)
Central Hospital Nykøbing Falster Denmark

UNUSUAL OCULAR MANIFESTATION IN TEMPORAL ARTERITIS

A case report

BY

NIELS VESTI NIELSEN and JENS SINDBERG ERIKSEN

Transitory oedema of the left orbit and maxillary sinus was revealed radiographically in an active phase of temporal arteritis This is pos[illegible] caused by inflammatory changes of the soft tissues in these regions Early signs of compromised retinal flow and anterior ischemic opt[illegible] neuropathy were demonstrated with fluorescein angiography This fin[illegible] promoted early immediate therapy

Key words temporal arteritis radiographically orbital oedema – fluoresc[illegible] – angiography – intraocular pressure

Ocular complications of temporal arteritis are frequent – 38–70 per c[illegible] (Parsons Smith 1959 Whitefield et al 1963) Sudden loss of vision is [illegible] means rare (20–72 per cent) owing to ischemic neuropathy of the op[illegible] or occlusion of the central retinal artery (Heptinstall et al 1954 Wa[illegible] Hollenhorst 1958 Hayreh 1975)

Less frequent and more atypical manifestations have been reported [illegible] muscle palsy visual hallucinations hypotonia bulbi and infarctions of [illegible] anterior eye segment – (Cullen & Coleiro 1976 Verdich & Nielsen 19[illegible]

No previous reports have described the appearance of radiograph[illegible] and maxillary oedema in temporal arteritis

Received November 16 1978

Case Report

ıan aged ɔ years was admitted to hospital on March 31 19,8 with a diagnosis of ınuous fever ESR 10ɔ mm/h and haematuria

ent History

patient had fever for 10 days prior to his admission
ınusitis was suspected and he was treated with antibiotics for seven days with no ›onse
urın the last six days before admission the patient had complained of a smarting burning pain of the tongue scalp pain frontal headache and intermittent left ıal disturbances in the form of rainbow vision

t History

history of eye diseases
The patient had been admitted in 19ɔ7 with haematuria for which no underlying se was detectable In 196? admitted with spondylosis of the cervical spine and in 3 with an injured back In 196ɔ the patient had a slipped lumbar disc
Ie had been well since then

rsical findings on Admission

neral condition was unaffected Blood pressure 140/,0 pulse rate 84 tmp 38 5°C
n the skull there was tenderness of left superficial temporal artery Both arteries re somewhat thickened but with good pulsation
ystoscopy of the urinary bladder disclosed one large and two small diverticula m which blood exuded. Puncture of the left maxillary sinus disclosed absence of usitis
Physical examination revealed no other pathological phenomena

hthalmologic consultation April 1 1978

st corrected visual acuity was almost 6/6 in both eyes
Visual field (campimeter) 3/1000 mm no scotoma nor defects
There was no protusion of the eyeballs The pupils reacted normally to light
Slit lamp examination disclosed dilated iris vessels of left eye Intraocular tension s 1ɔ mm Hg in both eyes with Goldmanns applanation tonometry
On ophthalmoscopy both papillae were normal Both retinae showed mild arterio erosis and incipient degenerative changes in the posterior pole
The ophthalmologist recommended biopsy of the temporal artery x ray examination sinuses and treatment with prednisone owing to suspicion of temporal arteritis

boratory Tests on Admission

b 7 0 mmol/l Leucocytes 13 3–10⁹/l ESR 43 mm/h Asp aminotransferase 50 U/l ⊢4ɔ) Creatinin 101 μmol/l Urine microscopy numerous erythrocytes Culture from ne streptococcus faecalis Serum protein quantitative immunologic Albumin 21 g/l

(34–53 g/l) Transferrin 1 0 g/l (2 0–2 7 g/l) Haptoglobin 5 3 g/l (0 7 ?1 g/l) Ig g/l (7 5–16 0 g/l) IgA 7 6 g/l (0 8–4 4 g/l) IgM 0 7 g/l (0 5–? 6 g/l) Orosomucoid (0 5–1 1 g/l) Complement C – 3 2 0 g/l (0 5–1 5 g/l)

We found negative titres on examination of AST and ASH rheumatic fa… no auto antibodies Cultures from blood were negative



Fig 1

X ray of the orbits and sinuses A blurring of the left orbit and maxillary … revealed during an untreated phase of ocular temporal arteritis

ay of sinuses no sinusitis but slight diffuse blurring of the left orbit and maxillary is (figure 1) Control radiography of the sinuses and orbits three days later showed blurring to have subsided

ay of thorax mild pulmonary emphysema Sequels of a fracture of left eight rib

G normal
After two days in bed there were no complaints of headache or visual disturbances rednisone treatment was postponed owing to the suspicion of an infectious disease l uncertainty regarding the diagnosis of temporal arteritis Results of biopsy of the superficial artery performed on April 2 19,8 was not available for 10 days
On April 1° the patient was referred to the Eye Department for correction of ding glasses The day before the patient had noticed intermittently blurring vision standing posture There was no concurrent headache Tmp was 3, S°C and E S R mm

ular State

ual acuity in the left eye was reduced to 6/15 best corrected
No tenderness to the temporal region was noticed but marked dilation of the blood sels of conjunctiva and sclera The pupils reacted normally to light
Slit lamp examination disclosed clearly dilated iris vessels of the left eye without ueous flare
Intraocular tension was 16 mm Hg on the right and 11 mm Hg on the left eye using oldmanns applanation tonometry
Ophthalmoscopy of the right eye was unchanged but in the left eye the peripapillary ion was characterized by slight blurring and the superior macular arterioles were closed by small exudates The venules were slightly dilated and darkened
Fluorescein angiography of the left eye revealed delayed filling of the retinal vessels d hypofluorescense of the optic disc In addition points of leakage in the centre of retina were seen (Fig 2)

eatment

ecadron (dexamethasone) 6 mg injected intravenously
Prednisone tablets 20 mg four times daily
Theophyllamine °00 mg injected intravenously
Intravenous theophyllamine drop 1 g/1000 ml of glucose over 24 hours Diamox *psules (acetozolamide)* 500 mg once daily
Histological examination was available the same day but after treatment had been stituted. It showed the left superficial temporal artery to have undergone changes mpatible with a diagnosis of temporal arteritis
Next day subjective improvement of general condition and vision had already curred Tmp 37 C
Left eye vision was improved to 6/, 5
The vessels of the conjunctiva and iris still were dilated and intraocular tension as 12 mg Hg in the right and 9 mm Hg in the left eye

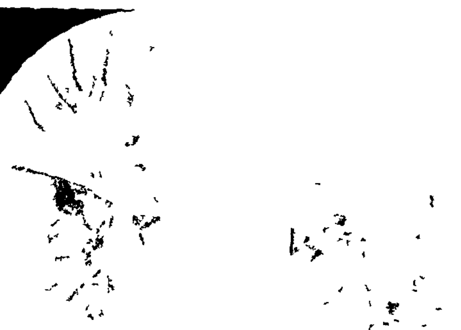


Fig 2 a b
Fluorescein angiography of the left eye The choroidal and retinal circulat slightly impaired (visual acuity 6/15) during untreated ocular temporal a – Fig 2 a Following treatment for one day circulation had been improved (visual 6/7 5) – Fig 2 b

Fluorescein angiography was repeated It showed an improved filling of the r vessels (Fig 2)

The patient was discharged on April 25 feeling well with no complaints and g visual acuity E S R 5 mm/h

Course

Monthly controls and two repeated fluorescein angiography examinations of t eye over the next four months showed no relapse

Discussion

A slight radiographically oedematous blurring of the left orbit a d max sinus was revealed in this patient The oedema may be assumed to con part of the exudative changes due to arteritis in the orbital and m – arteries

The orbital oedema remarkably did not cause protusion of the eyeball Both maxillary and orbital oedema were transient Thus the radiographical ring had disappeared after three days of confinement to bed without any lical treatment Moreover the general health was simultaneously improved se clinical findings probably indicate that the maxillary and orbital oedema his case was caused by temporal arteritis No previous reports are available these radiographic changes in temporal arteritis It is however a well wn fact that clinical signs of orbital involvement in arteritis temporalis be seen (Bettelheim 1968)

n our case confinement to bed had a favourable influence on the signs and nptoms of arteritis (visual disturbances and somatic complaints) These urred when the patient changed from a recumbent to an erect posture llenhorst (1967) had also observed a correlation between fluctuations of eye nptoms and postural alterations in temporal arteritis This phenomenon is bably due to a critical reduction of a marginal flow to the eye in the erect ture

As the left eye was involved during an aggravation of temporal arteritis a uction of the intraocular pressure of the affected eye was disclosed Hørven 73) accordingly has noted a reduction of intraocular pressure as a sign of ilar involvement in temporal arteritis in 22 patients Furthermore the pearance of hypotonia bulbi in temporal arteritis has been reported by ttelheim 1968 Daicker & Keller 1971 Goder 1968 Haimbok 1961 and rdich & Nielsen 1975

In our case an early fluorescein angiographic diagnosis of impaired retinal culation and an ischaemic optic neuropathy promoted immediate treatment

References

ttelheim A (1968) Die acute Ophthalmomalacia eine Folgersheinung der arteritis cranialis *Graefes Arch klin exp Ophthal 174* 359–366

illen J F & Coleiro J A (1976) Ophthalmic Complications of Giant Cell Arteritis *Surv Ophthalmol 20* 247–260

ucker B & Keller H H (1971) Riesencellarteritis mit endookularer Ausbreitung und Hypotonia bulbi dolorosa *Klin Mbl Augenheilk 158* 158–372

oder G (1968) Durchblutungsstorungen des Auges und biopsi der Arteria Temporalis G Thieme Leipzig

aimbock K (1961) Akute Hypotonia bulbi bei Sogenannter arteritis temporalis *Klin Mbl Augenheilk 138* 596

ayreh S S (1975) Anterior Ischemic Optic Neuropathy P 113–115 Springer Verlag Berlin Heidelberg New York

eptinstall R H Porter K A & Barkley H 1954) Giant Cell (temporal) arteritis *J Pathol Bacteriol 67* 507–519

Hollenhorst R W (1967) Effect of Posture on Retinal Ischemia Fro- ı- Arteritis *Arch Ophthal 78* 569-517
Horven I (1973) Dynamic tonometry V Further studies of the corneal ı- in temporal arteritis *Acta Ophthal (Kbh) 51* 353-366
Parsons Smith G (1959) Sudden blindness in cranial arteritis *Brit J O* 204-216
Verdich M & Nielsen N V (1975) Acute Transient Ophthalmomalacia a C Arteritis *Acta Ophthal (Kbh) 53* 875-878
Wagener H & Hollenhorst R W (1958) The ocular lesion in temporal arte *J Ophthal 45* 617-630
Whitefield A G W Bateman M & Cooke W T (1963) Temporal arteritis *Ophthal 47* 555-566

Authors address

Niels Vesti Nielsen M D
Department of Ophthalmology
Central Hospital
4800 Nykobing Falster
Denmark

stitut fur Physiologie und Biokybernetik der Universitat Erlangen – Nurnberg
(Direktor Wolf D Keidel)

›TIMULUS ALTERNATION AND FAST RETINAL POTENTIALS PHOTOPIC AND SCOTOPIC CONTRIBUTIONS*

BY

MATTHIAS KORTH and VIKTOR REIMAN

Human retinal oscillatory potentials in response to an alternating checker board pattern stimulus were studied in two subjects over an intensity range of 7.5 log units Under scotopic conditions two wavelets (S_1 and S) could be recorded At an intensity of 1 9 photopic log Td four high frequency oscillations (0_1–0_4) were noticed and a discontinuity was ob served in the corresponding luminance curve of the b wave together with a sudden decrease in the magnitude of the standard deviation of the amplitude measures The oscillations were noticed only on the ascending slope of the b wave With increasing stimulus intensity their latency de creased at a slower rate than that of the b wave and their number de creased Each wavelet had an amplitude maximum at a certain stimulus intensity level It was suggested that 0_1–0_4 were generated by the activa tion of the photopic *system* and that S_t and S were of scotopic origin

Key words averaged ERG – electroretinography – ERG wavelets – oscil latory potentials – pattern reversal – photopic ERG – rod cone break – scotopic ERG – stimulus alternation

ce the first description of the retinal oscillatory potentials by Cobb & Morton '54) many experiments have been concerned with their sites of origin and mechanism of generation Further much research has been concerned with question whether these oscillatory waves were triggered by the activity of rods or cones Results from several studies have indicated that the oscilla

*Mit Unterstutzung des Herrn Bundesministers des Inneren und des Bundesumwelt imtes

Received October 10 1978

... waves were probably of photopic origin In several experiments ... control the state of adaption oscillatory potentials in response ... flashes of light were obtained (De Molfetta et al 1968 Jacobson et al ... Algvere & Westbeck 1972 suggesting their photopic nature The ... experiments utilizing coloured light stimuli (Alfieri & Sole 19.. ... selective adaptation (Heck & Rendahl 1957 Rendahl 1958 19.. ... close relation of the oscillations with the photopic system Further ... in colour blind individuals no oscillations can be recorded (H ... 195. Goodman & Bornschein 1957) is also in line with these conclusions

In his clinical studies Algvere (1968a b) noted that in patients ... from disorders of the central retina the oscillations were more ... missing than in patients with diseases of the peripheral retina ... finding suggests that the oscillations are initiated by the cone system

Results of other experiments however seem to indicate that the ... system also contributes to the generation of oscillatory waves Auerbach ... after examining the wavelets during dark adaption ascribed some ... to photopic and others to scotopic activity Based on light and dark ... studies Algvere & Wachtmeister (1972) and Wachtmeister (1972 ... cluded that the contribution of the rods to the generation of the os... potentials could not be ruled out since they were most pronounced under ... pic states of adaptation

The somewhat contradictory conclusions from the experiments ... above may be due to the utilization of different procedures Also, the ... ledge of the state of the subject s adaptation seems to be very important ... order to judge the character of the oscillatory potentials

In the present experiment the method of stimulus alternation (J ... 1966) was used This procedure allows a good control of the eye ... since test and adaptation light are identical and the luminous flux ... pupil is kept constant throughout a recording session The stray light ... by conventional flashes of light is avoided thus leaving the ... response restricted to the directly stimulated retinal area Further a ... ber of sweeps were averaged in the present study in order to obtain ... responses at stimulus intensities lower than the intensity levels that ... pically been examined

Methods and Material

The stimulus consisted of a circular patch of a spatially alternating coloured check... board pattern presented in Maxwellian view The diameter of the visual ... 46 5° the size of one checkerboard element was 2 44 and a fixation mark ... in the center of the display

ntensity of the white light of a 75 Watt Xenon high pressure arc lamp (Osram) trolled by means of neutral density filters (Schott) The maximum retinal illu produced by the bright checkerboard elements as measured according to a re described by Westheimer (1966) using a digital photometer (Tetronix J16) photopic log Td for the lowest intensity level tested the corresponding value 63 scotopic Td

nethod of stimulus alternation has been described elsewhere (Armington 1968) present experiment an optical scanner (General Scanning G100PD) moved the board back and forth in a square wave fashion Each movement was completed 700 μsec and the alternation rate was two per second With each displacement stimulus pattern the total amount of light flux entering the pupil changed by 15%

electroretinogram (ERG) was recorded from the right eye with a silver electrode the eye by a scleral contact lens The right ear was used as reference the left e was grounded After amplification (Tektronix 122 and Tektronix 502 frequency se 0 2 Hz–10 kHz) the potentials were stored on an analog tape recorder (San model Sabre VI) together with the trigger signal of the square wave generator Hite model 5300 R) which drove the amplifier (General Scanning CCX 101) optical scanner Later the tape recorder was played back twice into a digital ter (PDP 11/40) and response averaging was performed with two different sweep s 512 sweeps were accumulated for each intensity setting and the responses to ack and forth movement were added together

experiments were carried out in an electrically shielded and light proof experi l chamber After a dark adaptation period of 30 min stimuli were presented in sing intensity in steps of 0 5 log units Stable head position was insured by means lental impression bite board Identical sessions applying the same sequence and r of intensities were repeated six times

healthy experienced male subjects (M K and V R) were used

v and fast ERG components were evaluated with sweep lengths of 495 ms and respectively In both cases the sweep comprised 512 points In order to isolate st components the short sweep records were digitally filtered so as to eliminate gh frequency components The smoothed function thus obtained was subtracted he unfiltered record thereby leaving only the fast potentials

compute the smoothed function a three point Hanning filter of the following thm was used

$$S(o) = \tfrac{1}{2}Y(o) + \tfrac{1}{2}Y(1)$$

$$S(i) = Y(i-1)/4 + Y(i)/2 + Y(i+1)/4$$

$$S(n) = \tfrac{1}{2}Y(n-1) + \tfrac{1}{2}Y(n)$$

S(o) represents the first data point

S(n) represents the last data point

S(i) represents the intermediate points that have been smoothed

Y(i) represents the value of the data point being smoothed

the 10 ms sweeps the above algorithm was repeated 1000 times resulting in an nuation of frequencies above 100 Hz by a factor of at least 100 (Fig 1)

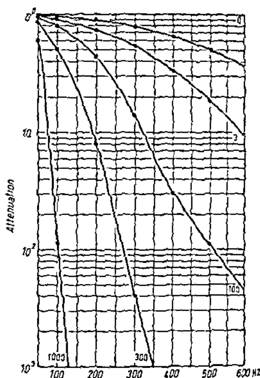


Fig 1

The degree of attenuation of the three point Hanning filter as a function of fre[quency]
The parameter is the number of repetitions of the filter algorithm Sweep len[gth]
number of data points was 512

The amplitude of the b wave was measured from the lowest point of the a wa[ve to] the peak of the b wave The amplitude of the high frequency components was de[fined] as the magnitude of the peak of a wavelet relative to the preceeding through a[nd] high pass filtered records

Results

Fig 2 presents three sets of ERCs collected from subject M K with increa[sing] stimulus intensities and with sweep lengths of 425 ms (a) and 40 ms (b and In b the smoothed b wave was plotted together with the original record Fo[r] shows the difference between the filtered and the unfiltered short sweep E[RG]

At the very low luminance levels (Fig 2 a) the response consisted ma[inly of] a slow negative going late receptor potential A b wave appeared at s[lightly] higher stimulus intensities Both the late negative potential and the b[wave]

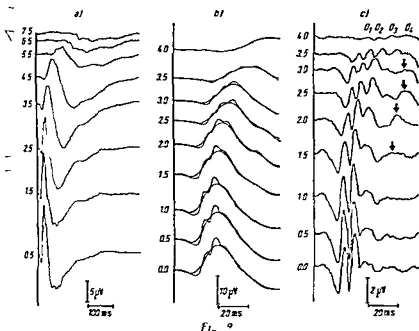


Fig. 9

filtered and filtered ERGs with two different time resolutions under different levels adaptation. The numbers along the ordinates indicate the values of the neutral den filters a) Unfiltered long sweep ERGs covering the whole intensity range tested. Photopic unfiltered and superimposed low pass filtered short sweep records c) High s filtered records obtained from b) by subtracting the low pass filtered from the iltered trace The arrows indicate a potential which was probably of scotopic origin. l traces are averages of six trials with a total of 30 2 sweeps The reversal of the mulus pattern took place at the vertical line shown at the beginning of each trace.

re superimposed by a few wavelets which became less distinctive as the mulus level was raised further Multiple waves resembling the typical oscil tory potentials began to appear again at the intensity given by the 4 0 neutral nsity filter (Fig 2 b c)

In Fig 3 the amplitude of the ERG b wave was plotted as a function of mulus intensity The resulting graph shows two important properties (1) here was a discontinuity in the curve with a plateau between the filter settings 0 and 3.5 (2) The magnitude of the standard deviation of the b wave am litude showing a steady increase with stimulus intensity abruptly diminished t filter 4 0 However with further increase in the stimulus level it increased monotonically again The discontinuity in the amplitude values and in the andard deviation suggested the presence of two different processes Thus in

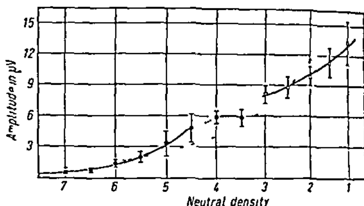


Fig 3

Luminance curve of the ERG b wave obtained from subject M K The da [illegible] averages of six trials The lower left and upper right smooth curves represe [illegible] tial fits to the filled and open circles respectively The two half filled [illegible] excluded from either curve fitting The dashed curve is an exponential fu [illegible] to all data points shown The vertical bars represent the magnitude of the [illegible] deviation

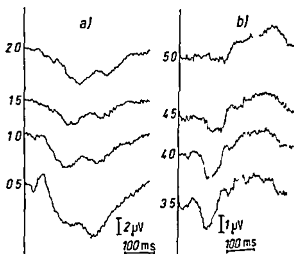


Fig 4

Averaged ERGs obtained with red light (a) and under foveal stimula [illegible] numbers indicate the values of the neutral density filters At filter set [illegible] 4 0 (b) the retinal illuminance was 2 0 and 1 9 photopic log Td respectively [illegible] represent averages of 1024 (a) and 2048 (b) sweeps

3 two separate exponential functions were computed and fitted to the data ts according to the method of least squares The filled circles of Fig 3 were for fitting a scotopic and the open circles were used for fitting a photopic e The half filled circles representing the plateau were not taken into ac t for any computation since they could not be ascribed satisfactorily to r process The dashed curve in Fig 3 was included to show that a single mential function could not accurately describe all the data points

1 order to determine the absolute threshold of the photopic b wave two itional experiments were performed using the right dark adapted eye of ct M K In the first one a red stimulus light of 670 mm (grating mono mator Schoffel model GM 250) with otherwise unchanged stimulus speci tions was used In the second experiment a circular stimulus of 4° in dia er containing a colourless checkerboard pattern with an element size of 25 centered on the fovea The original light beam without the monochromator used Fig 4 shows that a small b wave preceeding the negative after po ial could be distinguished for the red stimulus (a) at filter 1 0 and for the al ERG (b) at filter 4 0 The corresponding retinal illuminances were 2 0 1 9 photopic log Td respectively At those threshold intensities the b es obtained in the two experiments had identical peak latencies which in 1 were equal to the peak latency of the b wave obtained in the main ex iment at filter 4 0 (Fig 2)

he luminance curve obtained from subject V R had an almost identical pe and the discontinuity of the curve occurred at the same stimulus intensity

The amplitudes of the different fast components of the ERG shown in Fig 2 ibited a somewhat more complicated behaviour as intensity was raised The er half of Fig 5 shows that with increasing stimulus intensity the oscilla is increased in amplitude up to a maximum which occurred for each wavelet a different level of adaptation The amplitude of the smoothed b wave and steepness of a straight line between the lowest point of the potential wave ortly after stimulus onset and the highest peak of the b wave are shown for nparison in the upper half of Fig 5 The course of the amplitude of the first o wavelets resembled that of the amplitude and the steepness of the as iding slope of the filtered b wave From the agreement between amplitude d steepness of the filtered b wave it follows that the time to peak (as asured from the beginning of its onset) declined while its amplitude in ased (i e the spectral composition of the b wave shifted towards higher quencies with rising stimulus intensity)

Fig 6 shows that the peak latencies of the wavelets decreased with increasing mulus intensity at a slower rate than the peak latency of the smoothed b ave Across the intensity range during which the wavelets could be observed

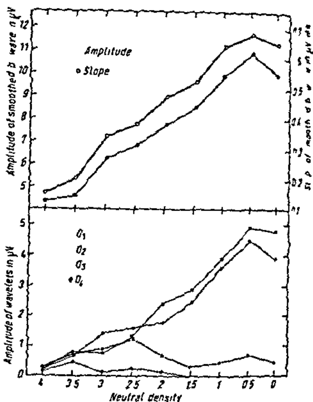


Fig 5

Upper part Amplitude and slope of the low pass filtered photopic ERG b wave as a function of stimulus intensity
Lower part Concurrent luminance curves of the four oscillatory wavelets

the average time between the first and the second wavelet was 6 4 ms, between the second and third 8 1 ms and between the third and fourth wavelet 9 1

The records shown in Fig 2 b indicated that the oscillations could be observed more easily when the initiation of a wavelet occurred simultaneously with the rising segment of the smoothed b wave As soon as the peak latency of the smoothed b wave became shorter than that of a particular wavelet, it started decreasing However the stimulus intensity could not be raised sufficiently that the peak of the b wave occurred earlier than the second or first wavelet (Fig 6)

Fig 7 shows superimposed original and smoothed traces (Fig 7a) as well as high pass filtered records (Fig 7 b) obtained under scotopic viewing conditions The smoothed records were obtained by repeating the digital filter algorithm 200 times at a sweep length of 425 ms At the very low intensities

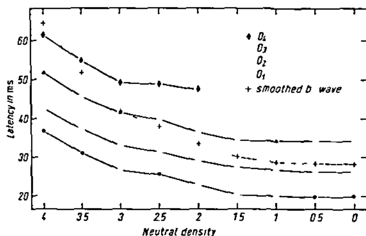


Fig 6

Peak latency curves of the four oscillatory wavelets and of the low pass filtered photopic ERG b wave as a function of stimulus intensity

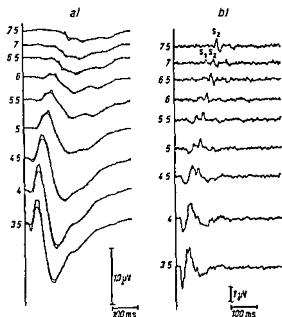


Fig 7

Unfiltered and filtered long sweep ERGs obtained under scotopic conditions The numbers along the ordinate refer to the values of neutral density a) Unfiltered and superimposed low pass filtered records b) High pass filtered records obtained from a) by subtracting the low pass filtered from the unfiltered traces Regarding wavelets S_1 and S refer to text Averages of six trials with a total of 3072 sweeps

wavelet (S) was superimposed on the early part of the late recept [illegible] At filter 7 0 the b wave appeared accompanied by another wavelet [illegible] preceeded S As stimulus intensity was increased the peak latencies of [illegible] waves became shorter the time span between them (average [illegible] amplitude did not change consistently At filter 4 0 it was impossible [illegible] a distinction between the b wave and S_1 wave S however could be [illegible] on the falling slope of the b wave as stimulus intensity was increased [illegible] Under photopic conditions its amplitude increased (Fig 2 c arrows) [illegible] intensity given by filter 2 0 and its waveform was composed of l[illegible] quencies than the high frequency oscillatory waves

The same number and a similar behaviour of the scotopic wavelets [illegible] found in subject V R

Discussion

The results of the present experiments clearly indicate that under both [illegible] and scotopic conditions wavelets could be recorded However the [illegible] spectral composition and dependency on the stimulus intensity were d[illegible] under the two conditions With increasing stimulus intensity typical osci[illegible] potentials were observed for the first time at an intensity of 1 9 phot [illegible] (density 4 0 in Fig 2) The discontinuity in the luminance curve of the b [illegible] accompanied by a sudden decrease in the magnitude of the standard dev[illegible] (Fig 3) occurring at this particular intensity could suggest that another [illegible] probably the photopic system might have dominated the response This [illegible] be in agreement with the results obtained by Armington et al (19[illegible] found that stimuli of 100 Td can be expected to yield photopic responses [illegible] nance curves that show a discontinuity separating a scotopic from a ph[illegible] branch have been obtained before using white (Goodman & Bornsch[illegible] or coloured stimuli (Korth & Armington 1976)

The view that the intrusion of the photopic system might be accompa[illegible] oscillatory potentials is supported by the records obtained with red [illegible] under foveal stimulation (Fig 4) It can be assumed that under those co[illegible] the responses were controlled predominantly by the activity of the cones [illegible] two experiments a b wave appeared at photopic intensities which were [illegible] identical with the intensity at which the discontinuity in the luminance [illegible] was observed

The plateau formed by the two half filled data points of Fig 3 [illegible] indication of a mesopic state of adaptation where an interaction betwe[illegible] scotopic and the photopic system takes place Thus it is suggested th[illegible]

ırrence of the oscillatory potentials (0_1–0_4) depends on the processes of the topic system No such high frequency oscillations were observed under sco c conditions However they may occur while both systems are active at the e time

n order to understand the mechanisms leading to the generation and sup sion of the wavelets the following three observations should be emphasized The occurrence of oscillatory waves on the ascending slope of the b wave noted by Bornschein & Goodman (1957) (2) Cobb & Morton s observation 54) of a faster decrease of the latency of the b wave than that of the wave suggesting the presence of two different mechanisms as assumed also by obson et al (1967) and by Wachtmeister (1973) (3) From observations 1 2 it can be concluded that the amplitude of the oscillations having a longer ncy than the peak of the b wave decreases to zero as was described by Ren l (1958) Genest (1964) Algvere & Wachtmeister (1972) and Tsuchida et al 73) These three observations were supported by the data of the present ex iment (Fig 2 b c) using an alternating stimulus The falling slope of the ave seemed to be associated with a mechanism damping the oscillations onset of this damping mechanism occurred with a shorter latency as sti lus intensity was increased The combination of these processes thus ex ins the different amplitude maxima of wavelets 0_3 and 0_4 (Fig 5)

he complete absence of oscillatory potentials in cases of diabetic retino hy (Yonemura et al 1962 Simonsen 1965 Nakajima and Sugimachi 1968) l the observation that the oscillations are more sensitive to the presence of a ease than the b wave (Algvere 1968a b) supports the notion that the b wave not triggered by or composed of oscillations The fact that oscillations have er been observed without the b wave indicates that the mechanism gene ing the wavelets depends in some way on the processes responsible for the neration of the b wave

The mechanism of origin of wavelets S_1 and S probably is different from that nerating the fast oscillatory potentials since they occurred under scotopic nditions and their amplitude and the time interval between the two wavelets l not depend on the stimulus intensity Also the waveforms of S_1 and S re composed of lower frequencies and S was observed on the falling slope the b wave Rendahl (1958 1964) regarded the fifth wavelet as being of otopic nature whereas Auerbach (1964) suggested that the first three wave s were of photopic and the fourth and fifth wavelet were of scotopic origin cording to the present results photopic oscillations and one scotopic wavelet curred at the same adaptation level with a considerable overlap of stimulus tensities

References

Alfieri R. & Sole P (1968) Adapto electroretinogram (AERG) in m... in man. In *The clinical value of electroretinography* pp 215-... Karger New York

Algvere P (1968a) Studies on the oscillatory potentials of the cl... gram *Acta ophthal (Kbh) Suppl 96* 4-85

Algvere P (1968b) Clinical studies on the oscillatory potentials of the ... retinogram with special reference to the scotopic b wave *Acta ophthal* ... 993-1024

Algvere P & Wachtmeister L. (1972) On the oscillatory potentials of the ... troretinogram in light and dark adaptation II Effect of adaptation to ... light and subsequent recovery in the dark A Fourier analysis *Acta* ... *50* 837-862

Algvere P Wachtmeister L. & Westbeck S (1972) On the oscillatory po... human electroretinogram in light and dark adaptation I Threshold and ... stimulus intensity on adaptation to short flashes of light. A Fourier anal... *ophthal (Kbh) 50* 737-759

Algvere P & Westbeck S (1972) Human ERG in response to double flashes during the course of dark adaptation A Fourier analysis of the oscilla... *Vision Res 12* 195-214

Armington J C (1968) The electroretinogram the visual evoked poten... luminance relation. *Vision Res 8* 263-276

Armington J C Marsetta R & Schick A M L. (1970) Stimulus alterna... level response *Vision Res 10* 227-236

Auerbach E (1964) The effect of slow intermittent light stimulation on the ... ERG *Docum ophthal 18* 376-391

Bornschein H & Goodman G (1957) Studies of the a wave in the human electro... gram. *A M A Arch Ophthal 58* 431-43

Cobb W & Morton H B (1954) A new component of the human electro... *J Physiol (Lond) 123* 36-37P

De Molfetta V Spinelli D & Polenghi F (1968) Behavior of electroretinogra... latory potentials during adaptation to darkness *A M A Arch Ophthal* ...

Genest A A (1964) Oscillatory potentials in the electroretinogram of the ... human eye *Vision Res 4* 595-604

Goodman G & Bornschein H (1957) Comparative electroretinographic ... genital night blindness and total color blindness *A M A Arch Ophthal* ...

Heck J & Rendahl I (1957) Components of the human electroretinogram ... in normal eyes and in color blindness Preliminary report *Acta physiol* ... 16 -1 5

Jacobson J H Hirose T & Popkin A B (1967) Oscillatory potential of the ... retinogram Relationship to the photopic b wave in humans *A M A Arch* ... *8* 58-67

Jacobson J H Hirose T & Popkin A B (1968) Independence of the osci... tial photopic and scotopic b waves of the human electroretinogram In *The clinical value of electroretinography* pp 8-20 Karger Basel New York

son E P Riggs L A & Schick A M L (1966) Photopic retinal potentials evoked phase alternation of a barred pattern In Burian H M and Jacobson J H Eds *nical Electroretinography* (Suppl 1 to Vision Res) pp 57-91
a M & Armington J C (1976) Stimulus alternation and the Purkinje shift *Vision* *s 16* 703-711
ujima A & Sugimachi Y (1968) Clinical value of ERG Metabolic disorders In *e clinical value of electroretinography* pp 243-249 Karger Basel New York
lahl I (1958) Components of the human electroretinogram The photopic electro inogram in normal eyes in deuteranopia and in deuteranomaly *Acta physiol ind 44* 189-202
lahl I (1964) The photopic components of the human electroretinogram Clinical ording with a slow flickering light stimulus *Docum ophthal 18* 344-351
ansen S E (1965) Electroretinographic study of diabetics Preliminary report *ta ophthal (Kbh) 43* 841-843
hida Y Kawasaki K Fujimura K & Jacobson J (1973) Isolation of faster com nents in the electroretinogram and the visually evoked response in man *Amer J ohthal 69* 19-24
htmeister L (1972) On the oscillatory potentials of the human electroretinogram light and dark adaptation IV Effect of adaptation to short flashes of light Time terval and intensity of conditioning flashes A Fourier analysis *Acta ophthal bh) 50* 950-969
htmeister L (1973) On the oscillatory potentials of the human electroretinogram light and dark adaptation III Thresholds and relation to stimulus intensity on laptation to background light *Acta ophthal (Kbh) 51* 95-113
theimer G (1966) The Maxwellian view *Vision Res 6* 669-682
emura D Aoki T & Tsuzuki K (1969) Electroretinogram in diabetic retinopathy *M A Arch Ophthal 68* 19-24

hor's address
Matthias Korth
itut fur Physiologie und Biokybernetik
Universitat Erlangen Nurnberg
20 Erlangen
versitatsstr 17
many

Department of Ophthalmology (Head Thore Ile Thomassen) Rikshospitalet The National Hospital University of Oslo

FAMILIAL CEREBRO MACULAR DEGENERATION (THE STENGEL-BATTEN MAYOU SPIELMEYER VOGT STOCK DISEASE) EVALUATION OF THE PHOTORECEPTORS

BY

EGILL HANSEN

In a material comprising seven patients with familial cerebro macular degeneration a grave visual disability had developed within a few years after the onset of the disease this being at 3½ to 7 years of age The night vision was only moderately rduced A severe red green colour vision defect was demonstrated in three patients This is in accord with the loss of red and green cone responses found in one patient by chromatic adaptation studies On the other hand a remarkably good response of the blue cone system was registered Normal pulse amplitudes were found by dynamic tonometry in four patients indicating good choroidal circulation This supports the theory that the degeneration of the neuroepithelium is of primary type The selectivity in loss of response functions being demonstrated here might however also be related to parallel degeneration taking place in ganglion cells

Key words juvenile amaurotic family idiocy – colour vision – dark adaptation – selective chromatic adaptation

Familial cerebro macular degeneration or juvenile amaurotic family idiocy neuronal ceroid lipofuscinosis with early manifestations in the eye The disease which typically begins at the age of 5–7 years usually leads to blindness at an early age Symptoms from the central nervous system appear and progressive demens and paralysis occur In most cases death follows before the age of years

Received November 23 1978

ı the Scandinavian countries the disease is known under the term Spiel er Vogt or the Spielmeyer Stock disease and in England as the Batten ou disease A better term is familial cerebro macular degeneration inas h as the eponyms of Batten (1903) and Mayou (1904) or Spielmeyer (1905) rt (1905) and Stock (1908) do not represent the first description of the ase An excellent description of the disease was already given in 1826 by ɔ Christian Stengel a general practitioner at Roros Norway in his report a curious disease in four siblings in the neighbourhood of Roraas Nissen ›4) drew attention to this early description of the disease

he incidence of the disease is about 1 50 000 of living children born (Ray 1969) being transmitted as an auto omal recessive trait In the Norwegian ister of the blind there are 19 known cases below the age of 15 years giving revalence in the same population group of 2 per 100 000

t is the purpose of the present article to report some patients examined at department with special emphasis on the findings which might serve to iracterize the affection of the photoreceptors at early stages of the disease

Material

the patiens suffering from familial cerebro macular degeneration are af ted at a young age extensive examinations are often impossible Besides the ntal disturbances which may be present the grave detorioration of vision y in itself reduce the patient to despair For instance in a 5 year old boy are of his reduced vision confrontation with colours which he was no longer e to see caused him to cry and the examination had to be broken off Certain aminations could be carried out on other patients However it is exceptional young children so influenced by their disease to be able to cooperate in aminations requiring their attention over a prolonged period Our patient 1 ppened to be such a patient The findings in the present report for the mos rt refer to this patient but are supplemented by findings obtained with the her patients The following patients were examined

tent 1 (C C) a girl was nearly 7 years old at the first examination There had been ual reduction during the last 1–2 years The visual acuity was finger counting 4 and n in the right and left eye respectively She had normal visual field limits but large ntral scotomas There was pathological pigmentation of salt and pepper type in the ndus and a Bull s eye appearance of the macula. The optic discs were slightly pale itially the vessels were normally calibrated The patient had experienced difficulties colour discrimination, especially red and dark were confused She also confused ht green and yellow as well as blue and purple During the observation period her ı twilight remained quite good

Course The visual acuity was gradually reduced At the age of 10 years ... see hand movements only in the temporal fields The atrophic changes ... were more pronounced and the vessels were distinctly thin. There was a reduction in the intellectual functions She became more apatic and slow ... 5½ years there had been two grand mal fits The patient was also examined at the Hospital for Sick Children London A rectal biopsy was taken and ... Mayou s disease

Patient 2 (T S) a girl had complained of eye problems since the age of 6 years ... slight reduction of visual acuity had been recorded Visual acuity was ... at the age of 7 years when she was admitted to the hospital Good visual fields were found by perimetry though with large central scotomas Mottled pigment ... found in the central fundus By an unconventional registration of dark adaptation slightly increased light thresholds were found during a 30 min dark adaptation ... A relatively better night vision than day vision had been noticed by the patient as well as by her parents This was the case even at an advanced stage of the disease From the age of 17 years the patient was forgetful and infantile Considerable ... tion had occured by the age of 21 years when she had had some epileptic fits ... dysphatic and unsteady

Patient 3 (K K) a girl was admitted to the hospital at the age of 7 years ... mental deviations There was a convergent squint predominately of the left eye ... acuity was 3–4/60 on the right eye and 2/60 on the left eye There was ... mentation in the macula and attenuated vessels

Patient 4 (F H) a girl had noticed visual reduction since the age of ... about 10 years of age she had noticed poor colour discrimination Red and ... confused like blue and white Her performance at low illumination was ...

When she was examined at the age of 14 years she could count fingers ... the distance of 1 m There was a constriction of the visual fields in the ... part and a great loss in the central area There was a greyish spot in the ... rounded by interrupted stripes and specks of pigment The fundus appeared ... and the vessels attenuated

She was mentally reduced at the age of 18 years by which time epileptic fits ... mal type had occurred The visual acuity was then reduced to light perception ... certain projection But even in this advanced stage she preferred low ... daylight

Patient 5 (H S) a girl was examined for the first time at the age of ... years ... had attacks of fury and stuttering from the age of 3½ years and there was a ... to stumble There was pigmentation in the fundus of salt and pepper type ... varied from 3/60 to 5/60 and was reduced to light perception at ... m only a ... 9½ years Her visual fields had been lost in the central and nasal part at the ... 8½ years

Patient 6 (E.B) a boy was nearly 7 years when examined Slight visual ... had been noticed during a half a year period when he also had been more ... Visual acuity was 6/40 on each eye The visual fields were constricted ... optic discs and attenuated retinal arteries were found There was atrophy of ... and fine mottled pigmentation in the central part of the fundus

asily confused colours especially red and green and also green and orange signals could not be seen except yellow which was quite easily recognized s were more easily seen with dark and white contrasts than with colour contrasts l clearly reduced vision in twilight though not pronounced At 7½ years of age ar after the first symptoms had appeared the visual acuity was finger counting at istance only in the temporal fields

(T E) a boy had noticed reduced vision since the age of years He was nable to discriminate red and green colours The recognition of blue colours was be good The visual acuity was 6/60 on the right eye and 6/36 on the left eye he was examined at the age of 8 years There was some salt and pepper pigmen in the central fundus In spite of some uncertainty by the perimetric examination orted good perception in the temporal and lower fields but significant loss in the nasal fields

Methods

ination of colour vision was performed with the following tests The AO HRR he Farnsworth s D 15 test the City University Colour Vision Test the Farns s tritan plate the Farnsworth Munsell 100 Hue test and the Sloan s achromatopsia A series of tissue paper contrast charts has been described earlier (Hansen 1970) olour vision tests were administered under two Macbeth Illuminant C lamps (300 A Nagel anomaloscope type I was used for the anomaloscope examinations imetric examinations during chromatic adaption were performed in a modified nann perimeter as has been described earlier (Hansen 19 4a Hansen & Seim Near monochromatic interference filters (half band width 10–15 nm) were used course of dark adaptation was registered with the Goldmann Weekers adapto by the integral adaptation method ERG responses to single flash and flicker li were recorded using an average technique Pulse synchronous variations in ocular pressure were registered by dynamic tonometry as described by Han)

Results

opic and scotopic light thresholds

standard static perimetry curve in patient 1 is depressed especially ral part (Fig 1) Dark adaptation was repeatedly registered at a of 8 years and showed a slightly flattened curve in the first se the final thresholds being about 1 log unit above the normal sensitivity during total dark adaptation shows a typical rhod 1) Static perimetry under scotopic conditions (Fig 2) demon moderate increase of absolute light thresholds in the central ed with the normal values differing by a little less than 1 r from about 25 in the periphery the light thresholds are

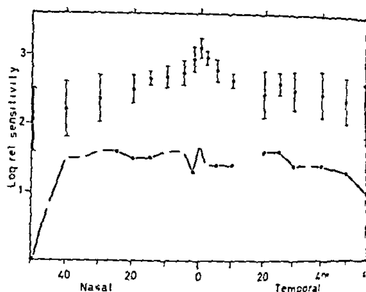


Fig 1
Static perimetry in standard illumination (10 cd/m²) registered in pat... size of target 54 (object IV) Mean ± 2 SD for 5 normals is indic...

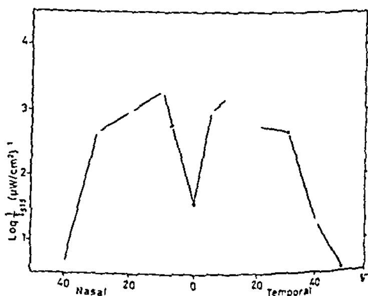
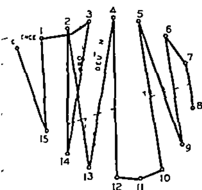


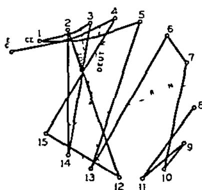
Fig 2
Static perimetry during total dark adaptation recorded with green object (... 515 nm) Ordinate absolute threshold sensitivity for target subtending ... meter Shaded area indicates the variation (mean ± 2 SD) for 5 ...

sed Dark adaptation registered in patient 7 at the age of 8 years showed a erate flattening of the curve the final thresholds being about 2 log units e the normal level Patient 4 who was examined at the Eye Department vål Hospital Oslo had dark adaptation curves at about the normal level

ur vision

performance of three patients with the Farnsworth s D 15 test is shown in 3 The confusions of patient 1 (CC) were mainly along the protan axis (re ered at the age of 7 years) Later on the confusion axes were more irregular





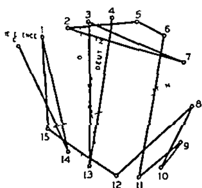


Fig 3
Performance of the Farnsworth s D 15 test for 3 patients

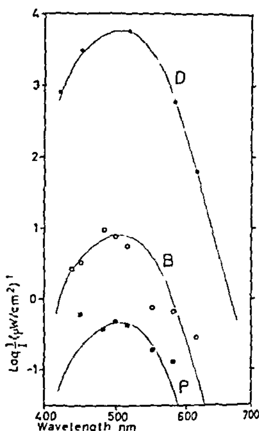


Fig 4

Spectral threshold sensitivity during total dark adaptation (D) in blue violet ba[ckground] (B) λ_{max} = 421 nm 3 lux and in purple background (P) Wratten 31A 3 l... records are 10 nasal central and 25 temporal for the three conditions ... respectively and angular size of targets 54 1°47 and 1 47 respectively Fitted ... nosity curves (after the I C E standard) are indicated

though clearly of red green type Patient 6 (FB) also showed an irregular ... tern though with dominance of confusions along the red green axes ... green defect was more clearly shown with the City University test where ... protan or deutan alternatives were preferred Patient 7 (TF) concentrated ... ly and had irregular confusions showing some preponderance along ... green axes The F D 15 test could not be performed by patient 4 ... reduced visual acuity like patient 2 who could only see coarse ... grey shades

With the OA HRR test patient 1 could see all the figures on the ... charts except on the screening charts and only one of the red green ...

ḥ the tissue paper contrast charts she was able to read the figures on the ts with blue yellow green and purple backgrounds in spite of her reduced ıl acuity On the other hand she could see none of the figures with green or red purple backgrounds Using the Farnsworth s tritan chart she could he green and not the blue square She had ‹11 error scores on the 100 Hue showing an irregular pattern though with less error scores in the red re

With the anomaloscope examination she could obtain matches over the re scale However the extreme red could not be seen Other red qualities • matched with dark yellow (‹0 on the red green screw with 3–5 and 68 ı 5) Extreme green was just brighter than the brightest yellow With the in s achromatopsia test red could be matched with a dark grey (3 5) while match could be obtained for the other colours

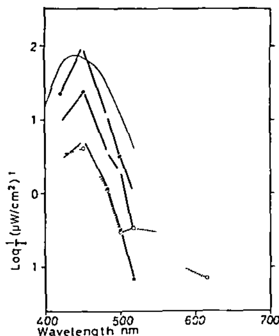


Fig ɔ

‹ ectral threshold sensitivity in yellow adapting light Wratten ⁹1 2300 lux at 7° na ‹l position (lower curve) compared with that of a normal person (age 21) obtained cen illy (stippled line) The upper and middle curves are obtained under reduced illumi tion (500 lux) at 7 nasal and 3 temporal positions respectively The curves are dis iced upwards and separated by 1/? log unit The smooth curve indicates the action spectrum of the blue mechanism (after Walraven 19‹4) Angular size of target 54

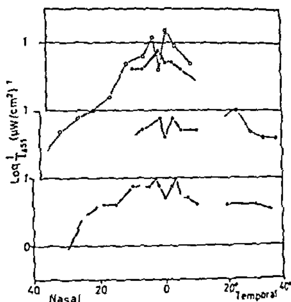


Fig 6

Static perimetry in yellow adapting light (Wratten 21) of varying intensities [illegible] (top) 1150 lux (middle) and 800 lux (bottom) Blue target (λ_{max} = 451 nm) [illegible] 34 visual angle is used Stippled line indicates the performance of a normal [illegible] (age 21)

Selective chromatic adaptation

In the purple adapting light favouring the green cone response pattern [illegible] not see any targets However at 25° peripheral position she could see [illegible] ject lights in reduced illumination (50 lux instead of 200 lux) Her [illegible] curve corresponds to the rhodopsin curve with an additional peak in the [illegible] which might be a contribution from the blue mechanism (Fig 4) In the [illegible] background a rhodopsin response curve is obtained by central [illegible] Thus during total dark adaptation as well as in the adapting purple [illegible] light her response curve is dominated by receptors of the rhodopsin type

In the yellow adapting light ($\lambda > 530$ nm) the patient's vision was [illegible] ingly poor in spite of the high illuminance value of 2 300 lux Conse[illegible] very large fixation marks had to be used The spectral peak sensitivity [illegible] yellow light was at about 451 nm showing a good blue cone response [illegible] Static perimetry performed in yellow light of varying intensity [illegible] target shows a good threshold sensitivity even in the central part [illegible] the same level as in a normal person (Fig 6)

ient 6 (at the age of 6 years) could clearly see a blue target (λ_{max} = ım) against a yellow background (Na light at λ = 589 nm 1300 lux) in the oral visual field However it was not possible to reliably register light holds By standard perimetry this patient was not able to see any red tar Another patient (7) indicated that he could not see any targets against a w adapting field

ro retinography

atient 1 ERG was present at the age of 6 years Reduced amplitudes cially low b waves were registered with single flash stimuli under scotopic itions Flicker registration (40 Hz) showed the same size of amplitudes gh with alternating peaks ERG was registered also at the Moorfields Eye pital London at the age of 7 years and showed reduced amplitudes At the



Fig 7
Fluorescein angiogram of patient 1 at the age of 7 years

age of 8 years the ERG was extinguished Patient 3 ... showed no response under scotopic conditions nor to slow ... patient 4 ERG was extinguished at the age of 14 years

Fluorescein angiography

The fluorescein angiogram obtained in patient 1 at the a e of ... moderate well limited fluorescence comprising an elliptic area ... region (about 10 diameter) The fovea itself is a normal da k ... No sign of leakage was registered in the present series of pic...

Dynamic tonometry

Corneal indentation pulse amplitudes were registered in four pat... amplitudes measured 20 μ in patient 1 36 μ in patient 2 31 μ in pa... 22–20 μ in patient 6 The patients were examined at the a e of ... 7 years respectively The results are within the normal values fo... (19 0) for the same age groups

Other findings

Vacuolized lymphocytes in the blood were found in high n... patients varying from 15% to nearly all of the lymphocytes be...

EEG was normal in patient 3 and in patient 1 in her first ex... later on slight irregularities were shown Irregularities indica... bral affection were found in the others

The initial neurological examinations revealed no pathological ... cept in patient 5 where instability of the lower extremities a d ... tonus of the muscles were found

Discussion

In familial cerebro macular degeneration a fundamental failure in th... oxidation metabolic inactivation which most likely affects certa... systems relatively early during the course of the disease Am... retinal rods and cones (Zeman 1974) Characteristically the d... debut with complaints related to central vision The age of onset ... was 3½ to ... years Visual disturbance was the only initial sym... patients 3 5 and 6 where slight mental deviations had also occ...

The degeneration of the neuro epithelium appears to be of th... as the histopathological examinations have always shown th... normal (Klien 1954 Hoffman 1956) The normal values found b...

of corneal indentation pulse amplitudes in our four patients indicate a good oidal circulation and is in accordance with this observation

pparently the degeneration of the neuro epithelium also involves the pig it epithelium as the fluorescein angiogram registered in patient 1 suggests istinct defect of the pigment epithelium in the macular region There is ably no defect of the Bruch s membrane as no leakage has been observed absence of ERG response in patient 3 as early as at 7 years of age and in ent 4 at the age of 14 years indicates a great disorder of the outer retinal rs Copenhaver & Goodman (1960) found absent or markedly depressed G in juvenile amaurotic family idiocy as opposed to a normal or borderline G in infantile amaurotic family idiocy In patient 1 ERG was present though uced at the age of 7 years At this early stage there was also flicker response ich suggests functioning cones Most probably this means blue cone activity possibly activity of peripheral cones However already after one year the G was absent

A characteristic feature in our patients was the rapid progression of the visual lure in many cases leading to practical blindness within a few years after onset of the disease Rather than a general constriction of the visual fields typical finding in our patients was the considerable loss in the central area advanced stages as was seen in patient 5 and 6 loss of the nasal field may o occur

As a curiosity of this kind of visual field loss a binasal hemiopi led to a misinter tation of the disease by Daae who in 1869 reported six cases which evidently were nilial cerebro macular degeneration Despite his description of typical retinal changes iae concluded that the disease must be related to affections of the visual pathways d not to the retinal changes

Colour vision has not usually been referred to in reports of familial cerebro acular degeneration probably because of the great visual reduction in those atients Oatman (1911) found central scotoma for green and red as one of the arliest manifestations Copenhaver & Goodman (1960) in one patient found npaired colour discrimination which could not be classified In a report of oizin et al (1962) of adult hereditary cerebro macular degeneration severe olour blindness was found in one patient a male having considerable visual oss and a further detorioration of vision in bright light The type of the colour efect was not specified

Typical red green colour defects were found in our patients The defects were ronounced already in the early stages Patient 1 evidently had an aquired red

green defect type I (after Verriest 1964) By the anomaloscope examination matches were of achromatic type suggesting no red or green receptors. This was confirmed by the chromatic adaptation experiments Only action spectra of rhodopsin type were obtained demonstrating here an example of scotopic type of vision (Fig 3) It is to be noticed that this patient was markedly [illegible] tated in yellow illumination containing only long wavelength light

A remarkable finding was the good response of the blue cone mechanism which was seen with patient 1 (Fig 4 and 5) The good perception of blue was confirmed by the colour vision tests Likewise in patients 6 and [illegible] a good perception of blue colours had been noticed

The retinal affection found in familial cerebro macular degeneration differs from that of retinitis pigmentosa where the blue mechanism is often [illegible] greatly reduced (Hansen 1977) There is also a clear difference concerning night vision which in retinitis pigmentosa is typically lost at an early stage. Relatively good night vision was indicated by our patients even in [illegible] stages of the disease This is consistent with observations referred by [illegible] (1911) in his survey including typical cases of maculocerebral degeneration.

Differential diagnosis may be difficult in the case of Stargardt's disease which in fact is a central cone dystrophy In Stargardt's disease the typical [illegible] likewise a colour vision defect of the red green type (François et al 1[illegible] distinct blue receptor response may also be shown (Hansen 1974 b)

Among familial cerebro macular degenerations individual differences occur However in our patients representing a common type in which the degeneration is chiefly central a selectivity was shown as to the [illegible] response functions It is difficult to explain why the blue mechanism is better preserved than the red and green mechanisms It may be that a [illegible] damage over a wider area of the retina is necessary for the blue [illegible] be lost Besides the primary degeneration taking place in the receptors degeneration is also in progress in the ganglion cells that is in the [illegible] elements The selective affection of the receptor responses probably [illegible] explained on the basis of both types of degeneration

Acknowledgments

The fluorescein angiography was performed by Dr T Flage and the [illegible] metry of patients 1 and 2 by Dr I Horven both of the Ophthalmological [illegible] Rikshospitalet The ERG examinations were carried out by Dr [illegible] Department of Clinical Neurophysiology Rikshospitalet All are gratefully acknowledged

References

F E (1903) Cerebral degeneration with symmetrical changes in the maculæ in members of a family *Trans ophthal Soc U K 23* 386–390

haver R M & Goodman G (1960) The electroretinogram in infantile late infan and juvenile amaurotic family idiocy *Arch Ophthal (Chicago) 63* 5о9–о66

A (1869) Hemiopi Det 6te Tilfælde i samme Slægt *Norsk Mag Lægevidensk 23* 6?1

ns J Verriest G & De Rouck A (1962) Heredo degenerescence maculaire juve avec atteinte prédominante de la vision photopique *Ann Oculist (Paris) 12* –1191

n E (1974) The colour receptors studied by increment threshold measurements ng chromatic adaptation in the Goldmann perimeter *Acta ophthal (Kbh) о?* 490–

n E. (1974) The photoreceptors in cone dystrophies *Mod Probl Ophthal 13* 8–3?7

sen E. (19/6) Examination of colour vision by use of induced contrast colours *Acta hthal (Kbh) о4* 611–6??

sen E. (1977) Investigation of retinitis pigmentosa by use of specific quantitative imetry II international visual field symposium Tubingen *Docum ophthal (De aa) Proceedings series 14* 461–4/?

isen E & Seim T (1978) Calibration of the Goldmann perimeter and accessories sed in specific quantitative perimetry *Acta ophthal (Kbh) о6* ?41–251

lman J (19 6) Pigmentary retinal lipoid neuronal heredodegeneration (Spielmeyer ogt disease) *Amer J Ophthal 4?* 1о–21

ven I (19 0) Dynamic tonometry *Acta ophthal (Kbh) 48* 23–38 39–58

n B A (19 4) Late infantile amaurotic idiocy *Amer J Ophthal 38* 470–4/5

)ou M S (1904) Cerebral degeneration with symmetrical changes in the maculæ in hree members of a family *Trans ophthal Soc U K 24* 14?–145

isen A J (19о4) Juvenil amaurotisk idioti i Norge *Nord Med о?* 1о4?–1о46

lman E. L (1911) Maculocerebral degeneration (familial) *Amer J med Sci 14?* ?1–23о

yner S (196?) *Juvenile Amaurotic Idiocy in Sweden* Diss University of Uppsala und 196? 107 pp

zin L Slade W Hermida H & Asao H (196?) Comparative histologic histochemi al and electron microscope studies of rectal biopsies in a case of adult hereditary cerebromacular degeneration In Aronson S M and Volk B W Eds *Cerebral sphingolipidoses A symposium on Tay Sachs disease and allied disorders* pp 57–72 Academic Press N Y

ngel C (18 6) Beretning om et mærkeligt Sygdomstilfælde hos fire Sødskende i Nærheden af Røraas *Eyr (Christiania) 1* 34 –3о?

ielmeyer W (190о) Ueber familiare amaurotische Idiotieen *Neurol Cbl ?4* 620–621

ock W (1908) Ueber eine bis jetzt noch nicht beschriebene Form der familiar auftre tenden Netzhautdegeneration bei gleichzeitiger Verblodung und uber typische Pig mentdegeneration der Netzhaut *Klin Mbl Augenheilk 46* ??о–244

erriest G (1964) Les déficiences acquises de la discrimination chromatique *Mem Acad roy Med Belg 2* ser 4 3о–3?

Vogt H (1905) Ueber familiare amaurotische Idiotie und verwandte Kr... *Mschr Psychiat Neurol* 18 161–1?1 310–357
Walraven P L (19?4) A closer look at the tritanopic convergence p... 1 ... 1339–1343
Zeman W (19 4) Studies in the neuronal ceroid lipofuscinoses *J Ne... rol* 33 1–12

Author s address
Egill Hansen MD
Department of Ophthalmology
Rikshospitalet The National Hospital
Oslo 1
Norway

Department of Ophthalmology (Head Aiyo Oksala)
University Hospital Turku Finland

CREENING OF RED GREEN DEFECTS OF COLOUR VISION WITH PSEUDOISOCHROMATIC TESTS

BY

EERO AARNISALO

Fifty red green defectives and 100 normal subjects were examined with the second edition of the Bostrom Kugelberg (BK II 1972) series and the Ishihara complete edition (1976) of pseudoisochromatic plates The results are related to those obtained with the first edition of the Bostrom Kugelberg test (BK I 1944) and the Bostrom test (II B 1950) and to the classification of defects obtained with the Nagel anomaloscope
The 50 red green defectives were originally selected by using a combination of the BK I and II B tests The normal subjects also passed this preliminary test as well as an examination with the Nagel anomaloscope
In the final examinations performed under standardized conditions three red green defectives passed both the BK II and the BK I test while eight defectives passed the Ishihara test Combination of BK II or BK I test with the Ishihara test does not improve the result Only one defective (a borderline case of protanomaly) passed the separate II B test
Normal subjects were not classified as colour defectives with any of the four pseudoisochromatic tests used All normal subjects passed both the BK II and the Ishihara test Classified as suspected red green defectives (one misreading made in standardized conditions) were five normal subjects with the II B test and one normal subject with the BK I test
In the second edition of Bostrom Kugelberg series the plates numbered 3 5 11 16 and 18 are clearly less effective than respective plates of the first edition. Only the plates numbered 1 and 10 have markedly improved in the second edition

Key words colour vision screening – pseudoisochromatic plates – red green defectives

ceived November 10 1978

Red green defectives made on average 0 54 misreadings per [illegible] BK II test as compared with respective 0 6[illegible] in BK I 0 69 in th[illegible] and 0 56 in the II B test

The first edition of the Bostrom Kugelberg pseudoisochromatic te[illegible] 1944) and the second edition (BK II 1972) both consist of [illegible] vanishing type showing figures of numbers (15 plates) or serpent[illegible] plates) or no figure at all (3 dissimulation plates) According to th[illegible] tions of this test persons making one misreading are suspected of [illegible] defective red green colour vision two misreadings indicate defective [illegible] vision while persons with normal colour vision are likely to pass the [illegible] without misreadings The BK I series is considered effective in dete[illegible] green defects (Kugelberg 1948 Chapanis 1948 Kettesy 1955 Belcher et al [illegible] Hansen 1963 Dreyer 1969) but some anomalous trichromats may [illegible] test without misreadings (Kugelberg 1948 Belcher et al 1958 Drey[illegible] Moreover the plates of the BK II test may be read correctly by so[illegible] malous trichromats (Hedin 1974)

The Ishihara test was first published in 1917 The latest two ed[illegible] the complete series (38 plates 1976) and the smaller series (24 pl[illegible] The plates of the complete series consist of two demonstration plates [illegible] with a number figure (8 plates of vanishing type 8 plates of transf[illegible] type 4 hidden digit plates and 4 diagnostic plates) and 1[illegible] pla[illegible] serpent line According to the instructions for this test the first 21 p[illegible] used to differentiate between normal and defective colour vis[illegible] making five to seven misreadings are suspected of having defective r[illegible] colour vision eight or more misreadings indicate defective colour vis[illegible] normals are likely to make maximally four misreadings There are [illegible] reports on effective differentiation between normals and red green d[illegible] obtained with the Ishihara test (Sloan & Habel 1956 Belcher et a[illegible] Crone 1961) Allowing 40 per cent of plates misread by normals e[illegible] differentiation between normals and red green defectives was obtained [illegible] the fifth (1925) seventh (1936) and ninth (1940) edition (Hardy et a[illegible] tenth (1951) edition (Katavisto 1961) and eleventh (1954) edition (Fr[illegible] Kettesy (1955) however found the tenth edition to be less effec[illegible] anomalous trichromats have been found to pass the Ishihara tes[illegible] Habel 1956 Belcher et al 1958 Dreyer 1969)

The Bostrom (II B 1950) test consists of 16 plates 15 of them d[illegible] figures of numbers the last one being a dissimulation plate The f[illegible] of this series is used only for demonstration thus only 14 plates a[illegible] ing type can be used for detecting red green defects According t[illegible]

s cf this test persons making one misreading are suspected of having ctive red green colour vision two misreadings indicate defective colour on while persons with normal colour vision are likely to pass this test iout misreadings The II B series is considered to detect red green defects ctively (Belcher et al 1958 Dreyer 1969)

he purpose of the present study was to investigate the effectivity of the nd edition (1972) of the Bostrom Kugelberg test and the last edition (1976) he complete series of the Ishihara test in screening of red green defects colour vision and to compare the results with those obtained with the first ion (1944) of the Bostrom Kugelberg series and the Bostrom (1950) test well as with the classification of defects obtained with the Nagel anomalo oe

Material and Methods

red green defective subjects were selected by testing 351 male students the university with plates of the BK I and II B series in combination The tes were shown in diffuse indirect midday daylight and subjects making or more misreadings were chosen for further examination These included ts in the following order with all plates of the BK I II B and BK II tests d with the first 25 plates of the Ishihara (1976) complete series Thus in Ishihara test the plates numbered 26 to 38 (serpent lines) were not shown e tests with pseudoisochromatic plates were performed with a Macbeth ecutive (BBX 324) daylight illuminator (effective illumination 1850 lux our temperature 7500 K) According to the instructions of the respective ts the plates of the Ishihara series were exposed for four seconds and the ates of the other tests were exposed for 15 sec All misread plates were own a second time Next followed an examination with the Nagel anomalo ope including measurements of the mid matching point and the matching nge The n range (the eye conditioned to the white light field of the omaloscope) and the u range (the eye conditioned to the bipartite coloured ld of the anomaloscope) were also measured (Schmidt 1955)

In the final series of 50 red green defective subjects (young men aged 19 36) the quotient of anomaly based on the mid matching point of the n nge varied between $\leq$ 0 70 and $\geq$ 2 0 An u range 10 scale units larger ian the n range was chosen to define extreme anomaly

All colour vision tests except the anomaloscope examination were per ormed binocularly The subjects used their regular eye glasses only all types f tinted glasses were excluded Subjects with myopia higher than 4 D were xcluded as were subjects with pathology of the eye fundi or a corrected isual acuity below 1 0

In the series of 100 normal young men (pupils of navig[illegible] 18 to 31) the quotient of anomaly was within normal limits [illegible] 0 72 and 1 28 The normal subjects passed all examinations as desc[illegible]

Results

Table I shows the number of plates misread by normal and red gree[illegible] subjects in the four different pseudoisochromatic tests ment[illegible] the BK II series normal subjects made no misreadings and th[illegible] defectives passed the test while two defectives both made one [illegible] In the BK I series one normal subject made one misreading three r[illegible] defectives passed the test and one defective made one misreading [illegible] 67 of the 100 normal subjects made no misreadings in the Ishiha[illegible] normals made one misreading three made two one made three [illegible] normal subject made four misreadings All red green defectives ma[illegible] readings in the Ishihara test two of them made only one mis[illegible] made two and four defectives made three misreadigns Fight red [illegible] fective subjects thus passed the Ishihara test Five normal subjects ma[illegible] misreading in the II B test but only one red green defective [illegible] this test without misreadings

Table II illustrates the effectivity in detecting red green defect[illegible] separate plates of the four pseudoisochromatic tests In BK II series th[illegible] numbered 6 and 10 are quite good Normal subjects made no [illegible] and only nine red green defectives (plate number 6) and respecti[illegible] defectives (plate number 10) read these plates correctly Less good [illegible] numbered 2 5 and 11 as shown by the fact that a high number of d[illegible] 49 45 and 44 respectively were able to read these plates correctly [illegible] series the plate number 2 also has a low effectivity All normal [illegible] 48 red green defective subjects read this plate correctly

The best plates of the Ishihara test are those numbered 6 and 8 All [illegible] subjects read these plates correctly but only seven red green defectiv[illegible] number 6) and nine defectives (plate number 8) read them correc[illegible] that as many as 28 normal subjects were unable to read correctly th[illegible] number 17 on the other hand no red green defective subjects was a[illegible] read this plate correctly Of an opposite kind is the effect of plate [illegible] Only one normal subject misread this plate but 39 defectives read it [illegible]

In the II B test one normal subject misread the plate number 5 [illegible] other hand only two red green defectives read it correctly Plate [illegible] is less good all normals passed but also 44 defectives read it correc[illegible]

Table 1

Number of plates misread in tests of Bostrom Kugelberg second edition (19[illegible]) Ishihara complete series (1976) Bostrom Kugelberg first edition (1944) and II Bostrom series (1950) by 100 normal subjects and 50 red green defectives

		Number of plates misread																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BK II 1972	normals	100																					
	defectives	3	[illegible]	[illegible]	2	2	1	4		1	2	4	6	6	7	8							
Ishihara 1976	normals	67	[illegible]8	3	1	1																	
	defectives		2	[illegible]	4		2		1		1	1	2	1			5	[illegible]	6	9	6	6	
BK I 1944	normals	99	1																				
	defectives	3	1	[illegible]	3	1	1	1	1	1	3	1	[illegible]	[illegible]	5	8	8	3	1				
II B 1950	normals	9[illegible]	5																				
	defectives	1		2	3	[illegible]	1	5	7	4	9	7	4	3	2								

Table II

Number of normal subjects misreading and number of red green defectives reading correctly the separate plates (serial number indicated) of tests of Bostrom Kugelberg second edition (1972) Ishihara complete series (1976) Bostrom Kugelberg first edition (1944) and H Bostrom eries (1950) Results obtained by 100 normal subjects and 50 red green defectives are shown

		Plate number in each series																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BK II 1972	normals																					
	defectives	96	41	19	25	45	9			12	5	44	14	24	19		22	17	33	15	16	
Ishihara 1976	normals									6							1	28	1	2	2	1
	defectives		15	25	18	15	7	10	9	[illegible]	16	12	14	18	15	14	10	0	13	24	28	39
BK I 1944	normals						1															
	defectives	34	48	9	19	35	11			13	13	27	11	[illegible]	12		10	10	16	18	17	
H B 1950	normals				1				1				9									
	defectives		30	29	11	27	35	33	[illegible]	15		35	15	16	11	44						

e l t (1944) d ll ıs st o ı c (30) by o u g
DA deute n aly EDA extreme de t ranomaly D deuteranopia PA pr tanomaly EPA extreme p tanomaly

		Number of objects	Number of plates misread																					
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BK II 1972	DA	20	2	1	2	1	2		1				1	2	4	1	3							
	EDA	17				1			2		1	1	2	4		3	3							
	D	5											1		1	1	2							
	PA	5	1	1				1	1			1												
	EPA	3													1	2								
Ishihara 1970	DA	20		1	2	3		1		1			1	1	1			2	1	2	2	2		
	EDA	17				1		1				1						1		2	3	3	5	
	D	5																		1	2	1	1	
	PA	5		1										1				1		1	1			
	EPA	3																1	1		1			
BK I 1944	DA	20	2	1	1	2	1	1	1					1	1	1	3	2	2	1				
	EDA	17								1	1	3	1		2	1	4	3	1					
	D	5													2	1	1	1						
	PA	5	1		1	1								1		1								
	EPA	3														1		2						
H B 1950	DA	20			2	3	1		3	1	2		3	1	3	1								
	EDA	17							2	4	1	6	3			1								
	D	5										2	1	2										
	PA	5	1				1	1				1		1										
	EPA	3								2	1													

Red green defectives made in average 9 1 misreadings 0.54 per separate plate in a total number of 17 plates (di simulation pl... of the BK II test as compared with respective 10.5 and 0 6? in the 13 9 and 0 69 in the 20 screening plates numbered ? to ?1 (demo diagnostic and serpent line plates excluded) of the Ishihara test a... 0 56 in the 14 plates (demonstration and dissimulation plates excl... II B test

The number of misreadings made in the four pseudoisochrom... different types of red green defectives classified with the Nagel a... are shown in Table III Note that some subjects with deutera... tanomaly made no misreadings or only a few misreadings wh... subject with extreme anomaly and no subject with dichromacy passed... a low number of misreadings

Also the diagnostic plates numbered 22 to 25 of the Ishihara test were ... both the normal subjects and the red green defectives of the present ... mal subjects read all these plates correctly Of the 50 red green defectives ... two or more misreadings in the diagnostic plates in these 3? cases (... per cent) qualitative diagnosis was correct in the rest of 18 cases the diagn... sible Of the anomalous trichromats of the present series qualitative diagnosis possible and correct in 16 cases but quantitatively all 16 cases were ... severe The matching range obtained with the anomaloscope (n range) ... units in one of these cases 4 scale units in two cases and less than 4 sca... the rest of 13 cases Obviously the quantitative diagnosis obtained with the ... test is unreliable and in many cases qualitative diagnosis cann t be made

Discussion

Proper use of a combination of the BK I and II B series of pseudoisochromatic tests provides an effective preliminary screening of red green defective ... vision (Chapanis 1948 Frey 1958 Katavisto 1961) Using this method ... screenings of the present study a high number (50) of red green d... were collected by examining 351 persons A relatively high rate ... red green defectives in this population may partly be due to the fact ... persons previously suspected to have defective colour vision may be likely to volunteer for the examinations On the other hand ... malous subjects may pass an examination with pseudoisochromatic tests ... berg 1969) In the present series the deutan/protan ratio of red green d... subjects was 5 25 as compared with 4 91 in a population examined in ... Finland with the Nagel anomaloscope (Forsius et al 1968)

ie protanomalous subject of the present series was able to read correctly lates of the BK I and II B series but he performed very slowly and ed a full 15 seconds per plate to pass these tests In the Ishihara test he one misreading only thus also passing this test His quotient of anomaly d on the mid matching point was 0 /0 the n range being 2 and the re 8 *scale units (the quotients corresponding to the limits of the u range* 0 59 and 0 91) and his match of pure red to yellow indicated abnormal nosity function in the red end of the spectrum Similar borderline cases rotanomaly have been described (Trendelenburg 1939 Heinsius 1975 e 19/2)

the present study normal subjects were not falsely classified as red green ctives with the BK II or Ishihara test Three red green defectives passed BK II test and two were additionally classified as borderline cases Eight green defectives pa sed the Ishihara test and three were classified as lerline cases Reducing the number of misread plates allowed in the ihara test from four to three does not alter the result Only three mis ings are allowed in Sweden in the official use of the Ishihara test (SosFS/ 977)

is recommended in the Ishihara test to note whether the person to be nined reads the hidden digit plates numbered 18 to 21 easier than the es of vanishing type numbered 10 13 14 and 17 In the present series procedure did not improve the result obtained with the Ishihara test

he three red green defective subjects who passed the BK II and BK I also passed the Ishihara test The eight defective subjects who passed Ishihara test all showed a narrow (n range less than 5 scale units) ano oscope matching range but an abnormal quotient In a large series of red n defective subjects there is a correlation between the number of errors le in pigment tests and the matching range obtained with the spectral its of the anomaloscope but this may not be true in individual cases lve 19/9) One of the deuteranomalous subjects of the present series mis d 13 plates in both BK II and BK I tests 18 plates in the Ishihara test and lates in the II B test but showed a matching range (n range) of 1 scale t only On the other hand one red green defective subject who passed Ishihara test showed in the anomaloscope examination a n range of one le unit but an u range of 16 scale units and was classified as a case of reme anomaly He made three misreadings in both BK II and Ishihara ts and seven misreadings in both BK I and II B tests

ites numbered 2 5 and 11 of the BK II series have low effectivity Only one l green defective subject misread the plate number 2 of the BK II series s was a case of extreme protanomaly making a total number of 13 mis

readings in this test In the second edition of the Bostrom K… the plates numbered 3 5 11 16 and 18 are less effective th… plates in the first edition of this test Only the plates numbered … the second edition have markedly improved Also the avera… misreadings as well as the number of misreadings per separa… by red green defective subjects is lower in the BK II series as c… the BK I series In BK I series the coloured spots forming the te… the spots of the background are printed on a slightly coloured b… the same hue as the spots of the background while the colours of … series are printed on a uniformly white background Saturation of … in the BK II series thus appears to be somewhat higher than in BK I…

The most effective separate plate of the four pseudoisochroma… appears to be the plate number 8 of the II B series One normal subj… this plate but only two red green defectives (one case of deu… one of protanomaly) passed Two deuteranomalous subjects who passed … and BK I tests both misread the plate number 8 of the II B te… they both misread the plate number 13 and one of them mis… number 10 The II B test may be somewhat difficult for normal… (Kettesy 1955 Frey 1958 Katavisto 1961 Heinsius 1963) In the … study five normal subjects (5 per cent) failed in this test (only … failed if plate number 12 is excluded)

Screening of red green defects of colour vision is usually made … bination of two reliable pseudoisochromatic tests (Frey 1958 Hein… In the present series of colour defective subjects combination of … obtained with the BK II or BK I test with those obtained with the I… test does not improve the result obtained with separate BK II or BK I… as the three red green defective subjects who passed both these te… passed the Ishihara test The separate II B test is more effective … only one red green defective subject passed This was the border… protanomaly described above

Acknowledgment

The author wishes to thank associate professor Valter Elenius for ad… tion and for the placing at his disposal of the Nagel anomaloscope … Sigrid Juselius Stiftelse

References

er S J Greenshields K W & Wright W D (1958) Color vision survey using Ishihara Dvorine Bostrom and Kugelberg Bostrom and American Optical rdy Rand Rittler tests *Brit J Ophthal 42* 355–359

om C G (1950) *Tabulae Pseudoisochromaticae* Kifa, Stockholm

om C G & Kugelberg I (1944 & 1979) *Tabulae pseudoisochromaticae B K* d and II ed Nordiska Bokhandeln, Stockholm

ams A (1948) A comparative study of five tests of color vision. *J Opt Soc ner 38* 676–649

e R A (1961) Quantitative diagnosis of defective color vision. A comparative aluation of the Ishihara test the Farnsworth dichotomous test and the Hardy nd Rittler polychromatic plates *Amer J Ophthal 51* 298–301

er V (1969) Occupational possibilities of colour defectives *Acta ophthal (Kbh) 47* 3–534

ius H Eriksson A W & Fellman J (1968) Colour blindness in Finland. 1 equency of protans and deutans 2 Family studies on minor deviations of the rmal mid matching point using the anomaloscope *Acta ophthal (Kbh) 46* 542–1

R. G (1958) Welche pseudoisochromatischen Tafeln sind fur die Praxis am sten geeignet? *Albrecht v Graefes Arch Ophthal 160* 301–320

sen E (1963) Factors causing uncertainty when conducting colour discrimination sts *An Inst Barraquer 4* 250–292

dy L H Rand G & Rittler M C (1945) Tests for the detection and analysis of lor blindness II The Ishihara test Comparison of editions *J Opt Soc Amer 35* 0–356

in A (1974) A study of the new series of Bostrom Kugelberg pseudoisochromatic es *Mod Probl Ophthal 13* 64–66

nsius E (1963) *Beurteilung des Grades der Farbensinnstorung bei verkehrmedi i ichen Gutachten Entwicklung und Fortschritte der Augenheilkunde* pp 614–623 erdinand Enke Verlag Stuttgart.

nsius E (1973) Die Abgrenzung der Farbentuchtigen und Farbenuntuchtigen bei er Untersuchung durch den Augenarzt *Klin Mbl Augenheilk 162* 75–80

nsius E. (1975) Die Grenzen zwischen den drei Formen der Trichromasie bei der Intersuchung am Anomaloskop *Klin Mbl Augenheilk 166* 87–94

lve J (1979) A comparative study of several diagnostic tests of colour vision used or measuring types and degrees of congenital red green defects *Acta ophthal (Kbh) Suppl 115*

hara S (1976) *The series of plates designed as a test for colour blindness* 38 lates edition. Kanehara Shuppan Co Ltd Tokyo

tavisto M (1961) Pseudoisochromatic plates and artificial light *Acta ophthal (Kbh) 39* 377–390

ttey A (19 5) Zur Beurteilung der Trennscharfe von pseudoisochromatischen Tafeln. *Klin Mbl Augenheilk 127* 156–163

gelberg I (1948) On the effectiveness of tabulae pseudoisochromaticae B K *Acta phthal (Kbh) 26* 429–437

gelberg I (1969) (In discussion) *Nord Med 82* 1457–1458

References

r S J Greenshields K W & Wright W D (1958) Color vision survey using Ishihara Dvorine Bostrom and Kugelberg Bostrom and American Optical dy Rand Rittler tests *Brit J Ophthal 4? 355–359*

m C G (1950) *Tabulae Pseudoisochromaticae* Kifa Stockholm

m C G & Kugelberg I (1944 & 19 2) *Tabulae pseudoisochromaticae B K* I and II ed Nordiska Bokhandeln Stockholm

inis A (1948) A comparative study of five tests of color vision *J Opt Soc er 38* 6?6–649

R A (1961) Quantitative diagnosis of defective color vision A comparative luation of the Ishihara test the Farnsworth dichotomous test and the Hardy id Rittler polychromatic plates *Amer J Ophthal 51* 298–307

T V (1969) Occupational possibilities of colour defectives *Acta ophthal (Kbh) 47* –534

us H Eriksson A W & Fellman J (1968) Colour blindness in Finland 1 quency of protans and deutans 2 Family studies on minor deviations of the mal mid matching point using the anomaloscope *Acta ophthal (Kbh) 46* 542– I

R G (1958) Welche pseudoisochromatischen Tafeln sind fur die Praxis am iten geeignet? *Albrecht v Graefes Arch Ophthal 160* 301–320

ien E (1963) Factors causing uncertainty when conducting colour discrimination ts *An Inst Barraquer 4* 250–29?

ly L H Rand G & Rittler M C (1945) Tests for the detection and analysis of lor blindness II The Ishihara test Comparison of editions *J Opt Soc Amer 35* 0–356

in A (1974) A study of the new series of Bostrom Kugelberg pseudoisochromatic s *Mod Probl Ophthal 13* 64–66

nsius E (1963) *Beurteilung des Grades der Farbensinnstorung bei verkehrmedi i schen Gutachten Entwicklung und Fortschritte der Augenheilkunde* pp 614–6?3 rdinand Enke Verlag Stuttgart

nsius E (1973) Die Abgrenzung der Farbentuchtigen und Farbenuntuchtigen bei r Untersuchung durch den Augenarzt *Klin Mbl Augenheilk 16?* 75–80

nsius E (1975) Die Grenzen zwischen den drei Formen der Trichromasie bei der Untersuchung am Anomaloskop *Klin Mbl Augenheilk 166* 87–94

ve J (19?) A comparative study of several diagnostic tests of colour vision used or measuring types and degrees of congenital red green defects *Acta ophthal bh) Suppl 115*

ihara S (1976) *The series of plates designed as a test for colour blindness* 38 lates edition Kanehara Shuppan Co Ltd Tokyo

tavisto M (1961) Pseudoisochromatic plates and artificial light *Acta ophthal (Kbh) 9* 3 7–390

ttesy A (1955) Zur Beurteilung der Trennscharfe von pseudoisochromatischen Tafeln *Klin Mbl Augenheilk 1?7* 156–163

gelberg I (1948) On the effectiveness of tabulae pseudoisochromaticae B K *Acta ophthal (Kbh) 6* 429–437

gelberg I (1969) (In discussion) *Nord Med 8?* 1457–1458

facility The rea on is that the possibilities for surgical ... uveoscleral pathways have not been investigated in any detail Th... scribes the results of our experimental study on the surgical ... uveoscleral outflow

Material and Methods

Six series of experiments were performed on 4? isolated cadaver eyes ... 36 h after death In all cases death was sudden In the first series we ... mea ure the scleral outflow facility in the others five surgical tech... lating of the uveoscleral outflow were evaluated

Scleral outflow facility measurement A needle (inner diameter ...) ... through the optic nerve into the eye The vitreous body was removed by ... suction The lens the retina and the uvea were destroyed and separa... of the eyeball by the sharp end of a needle and a tiny hook introd... needle With the help of the latter the large pieces of tissue were re... eye The tissue remnants were removed by irrigation and suction. The ... connected to a perfusion system which was filled with isotonic salt sol... The

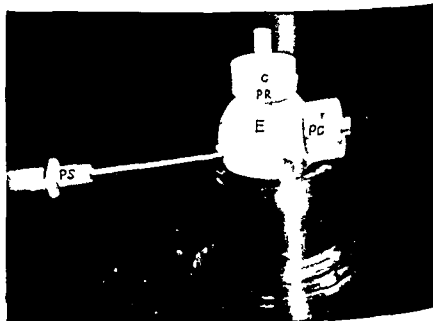


Fig 1

Scleral outflow facility measurements A needle which connected with pe... (PS) is inserted into the eyeball (E) A plastic ring PR) is fixed on the ... with ciacrin glue and ... ed with a cover (C) Conventional outflow pathways ... ... by a perilimbal suction cup (PC)

ı the system and ın the eye was maıntaıned at 20 mmHg With the help of an ıng mıcroscope the area of sclera free of perforatıng emıssarıes was found and d A plastıc rıng was placed on thıs area of sclera and fıxed wıth a synthetıc glue n SO 4) The ınner dıamter of the rıng was 9 mm the outer dıameter 12 mm and ıght ° mm Conventıonal outflow pathways were compressed wıth a perılımbal n cup The ınner dıameter of the cup was 11 mm the outer dıameter 13 mm and gatıve pressure ın the cup was 50 mmHg Then the scleral surface ınsıde the rıng rıed wıth a strıp of fılter paper and a cover was placed on the plastıc rıng ın or) elımınate evaporatıon of the fluıd (Fıg 1) After 15 mın the rıng was opened he fluıd whıch had gathered ın the rıng was removed and measured wıth the help mıcropıpet The procedure was repeated wıth the pressures ın the eye equal to 30 g and 40 mmHg The measuremens were made ın seven eyes

ıntatıon of a scleral strıp ınto supreuveal spase

ɾpodermıc needle connected to the perfusıon system was ıntroduced through the ı fırst ınto the posterıor chamber and then through the pupıl ınto the anterıor ber The needle meanıng the margın of the pupıl prevents deepenıng of the an ɾ chamber durıng perfusıon The outflow facılıty was measured at a pressure of mHg Two radıal ıncısıons ın the sclera were performed 3 to 5 mm from the cor lımbus The dıstance between the ıncısıons was 10 mm (Fıg 2) A spatula wıth an ıng at the end was ıntroduced through one ıncısıon ınto the suprauval space and :nd of the spatula was passed through the other ıncısıon A strıp of sclera 10 mm 2 mm wıde and about 0 5 mm thıck was excısed from another eye and one end of ıp was sutured The ends of the suture were ıntroduced ınto the op nıng at the ɔf the spatula and tıed The spatula was wıthdrawn and the scleral strıp was pull ıto the suprauveal space The strıp was placed parallel to the lımbus approxımately

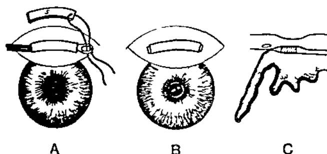


Fıg °

lantatıon of a scleral strıp ınto suprauveal space

\ spatula ıs ıntroduced ınto the suprauveal space through the radıal scleral ıncısıon and the end of the spatula ıs taken off through the other ıncısıon A strıp of sclera (SS) ıs excısed from another eye and one end ıs sutured The ends of the suture are threaded through the eye at the end of the spatula

The scleral strıp ıs placed ınto th suprauveal pace

The anterıor part of the suprauveal space ıs dılated wıth the scleral strıp

3 to 5 mm behind it The radial incisions of the sclera were closed with glue The outflow facility was measured for 10 min Eleven enucleated eyes were studied

Cyclodialysis In seven cadaver eyes an incision of sclera 3 mm long was made ... mm from the cornal limbus and parallel to the latter Through this incision cyclodialysis for a distance of 5 mm was performd with a spatula The incision was closed with a suture and the ciacrin glue The outflow facility was measured before and after closure of the scleral wound at a constant pressure of 20 mmHg

Implantation of a scleral strip into the supraciliary space combined with cyclodialysis The technique of the implantation of the scleral strip into the supraciliary space was the same as described above However before introducing the strip into the supraciliary space a cyclodialysis was performed for a distance of 5 mm through the radial incisions of the sclera Five eyes were studied with the help of this technique

Implantation of a scleral strip combined with sinusotomy After ... a rectoangular limbal based scleral flap 6 mm long and 5 mm wide was prepared (Fig 3) The flap included approximately 2/3 of thickness of the sclera The deep layer of sclera 5 mm long and 3 mm wide was excised and Schlemm's canal was opened The scleral strip was then introduced into the suprauveal space as described above The scleral flap was fixed with sutures and ciacrin glue The C values were measured before surgery and 10 min after this at a pressure of 20 mmHg 10 eyes were studied with this technique

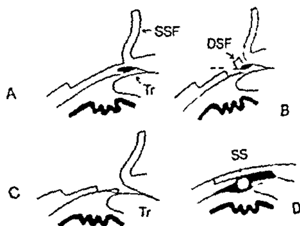


Fig 3

Implantation of a scleral strip combined with sinusotomy

A Superficial scleral flap (SSF) is prepared Note the position of Schlemm's ... the trabecular meshwork (Tr)

B Deep scleral flap (DSF) is prepared To open Schlemm's canal an incision ... along the dotted lin

C The deep scleral flap is excised and Schlemm's canal is opened

D The strip of sclera (SS) is placed into the anterior part of the ... The superficial scleral flap is fixed with sutures and ciacrin gl

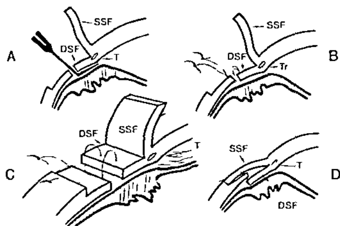


Fig 4

dialysis combined with goniospasis
uperficial (SSF) and deep (DSF) scleral flaps are prepared and cyclodyalisis is be g made
d C The anterior lip of the deep scleral incision is sutured with matress suture h ends of which are passed beneath the posterior lip and taken off at 1 mm be nd the incision
he mattress suture is tied up The superficial scleral flap is fixed with running ture

odialysis combined with goniospasis The procedure performed on six cadaver eyes as follows The eye was connected with the perfusion system A lamellar limbal l scleral flap 6 mm long and 5 mm wide was prepared (Fig 4) An incision of the scleral lamina at a distance of 4 mm from the corneal limbus and parallel to the r was made Cyclodialysis 5 mm long was then performed with a spatula The an r lip of the deep scleral incision was sutured with mattress suture both ends of h were passed beneath the posterior lip and passed through at 1 mm behind the in n At each side of the latter an additional incision of the deep scleral lamina was e They were directed towards the scleral spur The deep scleral flap thus outlined separated from the ciliary body With the help of the mattress suture a portion of deep scleral flap was pulled into the suprauveal space beneath the lip of the deep al incision The suture was then tied The external scleral flap was fixed with ing suture and completely closed with the glue The C values were measured be and 10 min after surgery

Results

eral outflow facility measurements For 15 min the mean amount of fluid ich flowed into the plastic ring was equal to 0 68 mm³ (range 0 23 to 1 3 ³) at a pressure of 20 mmHg 0 98 mm³ (range 0 30 to 2 0 mm³) at a pres

Table I

Surgical stimulation of the uveoscleral outflow in enucleated hu[illegible]
(mm³/min/mmHg) before and after surgery

Techniques	No of eyes	Initial C values		Final C values		[illegible]
		Mean	Range	Mean	Range	[illegible]
Implantation of scleral strip into suprauveal space	11	0 181	0 11–0.3[illegible]	[illegible]	0 1[illegible]–0 [illegible]	[illegible]
Cyclodilysis	7	0 1[illegible]4	0 11–0 23	0 [illegible]00	0 1[illegible]–0 2	[illegible]
Cyclodilysis + implantation of scleral strip	[illegible]	0 171	0 0[illegible]–0.30	0 2[illegible]4	0 1[illegible]–0 3	[illegible]
Sinusotomy + implantation of scleral strip	10	0 1[illegible]3	0 1[illegible]–0 2	0 [illegible]41	0 19–0 3	[illegible]
Cyclodyalisis + goniospasis	5	0 160	0 13 0 21	0 2[illegible]0	0 1[illegible]–0 [illegible]	[illegible]

The pressure in the perfusion system was 20 mmHg and the temp[illegible]
fusion solution was 20 C

sure of 30 mmHg and 1 40 mm³ (range 0 [illegible]4 to 3 1 mm³) at a p[illegible]
mmHg The area inside of ring was equal to 63 6 mm² One can [illegible]
the mean outflow facility coefficient was pressure independent [illegible]
0 0036 mm³ min/mmHg per 1 cm (100 mm[illegible]) of scleral surfa[illegible]

Implantation of scleral strip into suprauveal space The res[illegible]
Table 1 After surgery the mean C value increased by 0 041 ±0[illegible]
mmHg (mean ±SEM) This difference is significant ($P < 0$[illegible]
increase in the C value was 23 %, range 7 to 3[illegible] %. After rem[illegible]
from the suprauveal space the C value returned to the in[illegible]
even lower value

Cyclodialysis The mean increase in the outflow facility coeffic[illegible]
±0 015 mm³ min mmHg The C value increment varied fro[illegible]
averaging 30 %

Implantation of a scleral strip combined with cyclodialysis The [illegible]
in the C value was 0 103 mm³ min mmHg (ran[illegible] 0 0[illegible]–0 13) It [illegible]

to the sum of the mean C values increments in two previous series of ex ients C value increased in each case by 24%–96% (mean 60%)

intation of scleral strip combined with sinusotomy After surgery the mean lue increased by 0 071 ±0 014 mm³/min/mmHg The C value increment d from 18 to 71% averaging 41%

odialysis combined with goniospasis In each of five cases the coefficient ow facility increased by 0 06 to 0 14 (mean 0 10) mm³/min/mmHg As ared to its initial level the C value increment varied from 43% to 108% n 67%)

Discussion

uveo scleral outflow route consists of three parts the anterior portion of iliary body the suprauveral space and the sclera Each part exerts a cer resistance to the aqueous outflow

follows from the first series of our experiments that the rate of flow igh the sclera is directly proportional to the outflow pressure If the latter ual to the average normal intraocular pressure (15 mmHg) and the whole r scleral surface (1400 mm²) is available for the aqueous 35% to 40% of outflow (2 mm³/min) passes through the sclera It is approximately twice iuch as was reported by Fatt & Hedbys (1970) We admit that in our ex ments tiny emissaries could be presented in the areas of sclera under in igation Taking into consideration the real existance of the perivascular es in the sc era it is supposed that our data are not far from reality How r it was demonstrated by Bill & Phillips (1971) that the uveo scleral route responsible for only 4% and 14% respectively of the total outflow in human eyes

hus a considerable part of the uveo scleral outflow resistance seems to be ted in the uveal and suprauveal portions of the uveo scleral pathways The is ance of the first portion can be diminished by cyclodialysis and the ond portions with the help of a widening of the suprauveal space The ter appears to be only a potential space It was found that there is a con erable resistance both to circumferential and meridional flow of fluid in the rauveal space (Cherkasova 1976)

To widen the suprauveal space a strip sclera was placed in the anterior rtion of the space Both cyclodialysis and implantation of a scleral strip ve approximately the same results the mean C value increased by 0 04– 05 mm³/min/mmHg Cyclodialysis combined with implantation of a scleral ip resulted in a significantly larger increase of the outflow facility coef

Department of Ophthalmology (Head F Wester)
Central Hospital Nykøbing Falster Denmark

TIMOLOL
TRANSITORY MANIFESTATIONS OF DRY EYES IN LONG TERM TREATMENT

BY

NIELS VESTI NIELSEN AND JENS SINDBERG ERIKSEN

In 64 patients treated with timolol eye drops (0.25% and 0.5%) [illegible] patients developed transitory sensation of dry eyes. Two of these [illegible] also had xerostomia. Conjunctival and corneal defects were [illegible] simultaneously with rose bengal staining. Morphologically some of the lesions might have the same appearance as of the early stages of [illegible] conjunctivitis sicca. A reduction of Schirmer test and break up t[illegible] was noted. The duration of symptoms ranged from 5 to 13 days. The [illegible] time of treatment at the debut of symptoms was 30 weeks ([illegible] weeks). A pathogenesis of these seemingly harmless find[ings] [illegible] present obscure.
In none of our patients was the treatment discontinued and the [illegible] did not reappear.

Key words: timolol – adverse effect – transitory – dry eyes – rose bengal staining dots

Timolol maleate, a non selective beta blocker, has been demonstrated [illegible] a potent hypotensive effect on an elevated intraocular pressure [illegible] used topically (Katz et al 1976, Kerts & Horven 19[illegible], Knerr[illegible] Nielsen 1978, Radius et al 1978, Ritch et al 1978, Zimmerman & [illegible] 19[illegible]). The future role of timolol in the treatment of glaucoma [illegible] present time to be very promising.

To day no local or systemic significant adverse effects in the [illegible] long term use of timolol ophthalmic solution have been reported [illegible]

Received January [illegible] 1979

ar complaints (transient burning smarting pain and slightly blurred vision) been noted previously (Nielsen 1978 Radius 1978 Preclinical Brochure imolol 1977)

ractolol and oxprenolol have induced serious ocular adverse effects – omucocutaneous syndrome – when administered systemically (Felix et al 1 Garner & Rahi 1976 Holt & Waddington 1975 Wright 1975)

1 a long term study we have observed transitory superficial lesions of the junctiva and cornea together with the sensation of dry eyes in seven patients ted with timolol eye drops

Materials and Methods

material consisted of 64 patients 3 women and 27 men, with an increased IOP mean age was 66 3 years (ran e 30–85 years) Ten were treated with timolol eye ps 0 25 % × 1 and 54 with 0 50 % × 1–2 The subjects were regularly controlled 1 5 9 15 and 27 weeks from the start of treatment. Thereafter with an interval of weeks

The mean time of observation in the present material was 38 weeks ranging from to 57 weeks

With the exception of one person with rheumatoid arthritis no patients suffered m any form of collagenosis

At control visits corneal ensitivity with Cochet & Bonnet aesthesiometer (Norn 4) and rose bengal vital staining of the conjunctiva and cornea were recorded tially

ichirmer test I then was investigated (Norn 19 4) When the symptoms of d– peared in this material break up time measurement BUT

ded compared with 25 subjects w–

Thereafter mea–

Department of Ophthalmology (Head E We els Central Hospital Nykobing Falster Denmark

TIMOLOL
TRANSITORY MANIFESTATIONS OF DRY EYES IN LONG TERM TREATMENT

BY

NIELS VESTI NIELSEN AND JENS SINDBERG ERIKSEN

In 64 patients treated with timolol eye drops (0 25 % and 0 [illegible] patients developed transitory sensation of dry eyes Two of these [illegible] also had xerostomia Conjunctival and corneal defects were [illegible] simultaneously with rose bengal staining Morphologically some of the lesions might have the same appearance as of the early stages of [illegible] conjunctivitis sicca A reduction of Schirmer test and break up time [illegible] noted The duration of symptoms ranged from 3 to 13 days The time of treatment at the début of symtoms was 30 weeks ([illegible] weeks) A pathogenesis of these seemingly harmless findings [illegible] present obscure
In none of our patients was the treatment discontinued and the [illegible] did not reappear

Key words timolol – adverse effect – transitory – dry eyes – rose bengal staining dots

Timolol maleate a non selective beta blocker has been demonst[illegible] a potent hypotensive effect on an elevated intraocular press[illegible] used topically (Katz et al 1976 Kerty & Horven 19,8 Krieg[illegible] Nielsen 1978 Radius et al 1978 Ritch et al 19,8 Zimmerman [illegible] 19,,) The future role of timolol in the treatment of gla[illegible] present time to be very promising

To day no local or systemic significant adverse effects in [illegible] long term use of timolol ophthalmic solution have been rep[illegible]

Received January ? 19)

r complaints (transient burning smarting pain and slightly blurred vision) been noted previously (Nielsen 1978 Radius 1978 Preclinical Brochure imolol 1977)

actolol and oxprenolol have induced serious ocular adverse effects – mucocutaneous syndrome – when administered systemically (Felix et al Garner & Rahi 1976 Holt & Waddington 1975 Wright 1975)

a long term study we have observed transitory superficial lesions of the unctiva and cornea together with the sensation of dry eyes in seven patients ed with timolol eye drops

Materials and Methods

naterial consisted of 64 patients 37 women and 27 men with an increased IOP nean age was 66 8 years (range 30–85 years) Ten were treated with timolol eye 0 25 % × 1 and 54 with 0 50 % × 1–2 The subjects were regularly controlled 1 5 9 15 and 27 weeks from the start of treatment Thereafter with an interval of eeks

e mean time of observation in the present material was 38 weeks ranging from 52 weeks

ith the exception of one person with rheumatoid arthritis no patients suffered any form of collagenosis

control visits corneal sensitivity with Cochet & Bonnet aesthesiometer (Norn) and rose bengal vital staining of the conjunctiva and cornea were recorded illy

hirmer test I then was investigated. (Norn 1974) When the symptoms of dry eyes ared in this material break up time measurement BUT (Norn 1974) was further d compared with 25 subjects without symptoms and signs of dry eyes

ereafter measurements of IOP with Goldmanns applanationstonometer the dia r of the pupil pulse rate and blood pressure were performed

rum levels of haemoglobin leucocytes immunglobulins (IgG IgM IgA) acute e proteins Rose Waaler RAT ANF and smooth muscle cell antibody were con ed in the subjects with symptoms and signs of dry eyes compared with 23 omly selected timolol treated subjects without symptoms of dry eyes

stical procedure

statistics were carried out by means of Mann Whitney ranksum test and chi re test.

Results

our series seven patients four women and three men appeared to have dry s and abnormal vital staining of the conjunctiva and cornea with rose gal dye Two of these patients moreover complained of xerostomia The

mean age of the patients was 72 years (range 63–78 years). The dry eyes together with slight soreness and xerostomia were ... by the patients. One patient had some complaints of dry eyes ... changes before treatment with timolol. The symptoms and ... appeared after a mean treatment period of 30 weeks (range ...). mean duration of symptoms was seven days (range 3–13 days). ... were treated with timolol 0.25 % × 1 and the remaining ... 0.50 % × 1–2. The mean IOP was 18.7 mm Hg (± 0.46 SEM) in the ... 19.7 mm Hg (± 0.73 SEM) in the left eye. None of these subjects had ... of rheumatic disease or any other collagen disease before treatment ... timolol.

In one male patient antiglaucoma surgery (trabeculectomy) on ... had previously been performed.



Fig. 1

...ar old w... with sicca symptoms. Rose bengal vital dye ... tate and one confluent staining of cornea.

Ocular findings

Bengal vital staining

rose bengal staining slit lamp examination disclosed numerous staining on the medial and lateral bulbar conjunctiva and in varying degress ate stippling on the cornea (Fig 1) These changes were mainly localised e interpalpebral fissure In the bulbar and tarsal conjunctiva a slight remia was noticed There was no sign of any intraocular reaction In th ee cts only one eye was affected

eal sensitivity (Cochet & Bonnet s aesthesiometer diameter 0 12 mm) patients with dry eyes a slight tendency towards reduced corneal sensi was observed (mean reduction 6 mm range 5–10 mm) compared with ous measurements ($P > 0.05$)

k up time (B U T) In the affected eyes the mean BUT was 7 1 seconds e 5 to 9 seconds) In 23 persons without dry eyes measurement of BUT ared to be 12 1 seconds (range 5 to 25 seconds) This difference was ficant ($P < 0.01$)

Table 1

The distribution of increased serum levels of serum proteins and autoantibodies in seven subjects with and 23 subjects without dry eyes

Serum proteins and autoantibodies	+ Dry eyes (n 7)	– Dry eyes (n 23)
I g G	1	
I g A	1	
I g M		
Orosomucoid		2
Complement (C 3)	3	2
Haptoglobin		1
Transferrin	1	4
Antinuclear antibody		
Smooth cell antibody		
LE cell Test		
Waaler Rose		1
R A T		

gnificant increase $P < 0.05$ (Chi square test)

Schirmer test As symptoms appeared the mean values of [illegible] were significantly reduced to 6 1 mm/5 minutes ranging from [illegible] ($P < 0.01$) Previous values obtained showed mean 11 2 mm (ra[illegible] In subjects without dry eyes Schirmer test was mean 14.3 (ra[illegible]

Blood samples The number of subjects with increased serum lev[illegible] are illustrated in Table 1 There was a tendency towards increased [illegible] serum complement (C 3) in the group with dry eyes (43 %) [illegible] subjects without symptoms (9 %) It is noted that none of the [illegible] subjects had positive values of RAT smooth muscle antibody or [illegible]

No systemic adverse effects were noted Pulse rate and blood p[illegible] unchanged at controls No change in pupillary size was observed [illegible] the seven subjects did symptoms of dry eyes reappear

Discussion

At the present time no other reports dealing with the appearance of [illegible] and superficial lesions of cornea and conjunctiva during treatme[illegible] eye drops are available Laboratory studies have failed to reveal [illegible] ocular toxity with timolol ophthalmic solution (Preclinical Broch[illegible] 1977) However transient superficial keratitis has been reported in [illegible] without unravelling the mechanism of this manifestation

In our cases it is noteworthy that the symptoms of dry eyes [illegible] approximately the same time The subjects spontaneously compla[illegible] eyes and simultaneously both functional and morphological signs of [illegible] lacrimal secretion were disclosed These manifestations were [illegible] only present for a short time It should be stressed that only [illegible] subjects had suffered from dry eyes before treatment with timolol [illegible] no ocular or systemic disease which could explain these probable [illegible] of sicca manifestations However our clinical findings need ca[illegible] tions We lacked a placebo treated control group and have not [illegible] mination of tear proteins

The pathogenic mechanism responsible for the present [illegible] difficult to unravel Adverse ocular reactions (oculomucocu[illegible] have been induced by practolol and oxprenolol when used [illegible] & Waddington 1975 Wright 1975) Furthermore cutaneous side [illegible] propranolol have been described (Jensen et al 1976) Immuno[illegible] nisms have been suggested to explain both ocular and cutaneo[illegible] tions to these beta adrenergic blocking agents Amos et al 19[illegible]

Garner & Rahi 1976 Jensen et al 1976 Mackie et al 1977 Wright
ı No ocular toxity of practolol has been reported when administered
ally to the eye (Vale & Phillips 1973) In our cases we found a tendency
creased levels of serum complement (C 3) but this finding is far from
usive Low serum levels of ocular applied timolol have been demonstrated
linical Brochure on Timolol 1977) However ocular symptoms have not
when timolol has been administered systemically (Mackie et al 1977)
the sympathetic nerve supply of the lacrimal gland has not
xplained (Adler 1970) However it may be suggested
blocking agents affect the lacrimal secretion by an
pathetic innervation of the gland A local untoward
film in some predisposed subjects could be a possible
ol induced dry eyes We have not obtained analysis of
mmunologic investigations of conjunctiva tear flow fluor
m levels of timolol in our cases These procedures will be
future if symptoms reappear with the hope of clarifying
ıl manifestations of dry eyes during treatment with timolol
n

Acknowledgment

this study was kindly supplied by Merck Sharp & Dohme

References

1970) Physiology of the Eye 5th ed rev R A Moses Mosby USA pp 24

Birgden W D & Mc Kerron R A (1975) Untoward effects associated
tolol Demonstration of antibody binding to epithelial tissue *Br Med J 1*

I Ive F A & Dahl M G C (1974) Cutaneous and ocular reactions to
ol *Br Med J 4* 391

\ & Rahi H S (1976) Practolol and ocular toxity Antibodies in serum and
Brit J Ophthal 60 684

J A & Waddington E (1975) *Oculocutaneous reaction to oxprenolol Br Med*
539

en H A Mikkelsen H I Wadskow S & Søndergård J (1976) Cutaneous reactions
propranolol (Inderal) *Acta Med Scand 199* 363

I M Hubbard W A Getson A J & Gould A L (1976) Intraocular pressure
crease in normal volunteers following timolol ophthalmic solution. *Invest Ophthal*
15 459

& Horven I (1978) Glaucoma treatment with timolol *Acta Ophthal (Kbh)*
55

Kriegelstein G K (1978) Die Wirkung von Timolol Augentropfen a f ... innendruck bei Glaucoma simplex *Klin Mbl Augenheilk* 1 (
Mackie I A Seal D V & Prescod J M (1977) Beta ad energ c re... drugs tear lysozyme and immunological screening for adverse r ... *Ophthal* 61 354
Nielsen N V (1978) Timolol Hypotensive effect used alone and in c... treatment of increased intraocular pressure *Acta Ophthal (Kbh)* ...
Nielsen N V (1979) Timolol Long term use for treatment of increased ... pressure (In prep)
Norn M S (1974) *External Eye* Method of Examination. Scriptor Cope... 99 133
Preclinical Brochure on Timolol (1977) Merck Sharp & Dohme Rese... West Point Pa
Radius R L Diamond C R Pollack I I & Langham M F (19... Te... drug for management of chronic simple glaucoma *Arch Ophthal* ...
Ritch R Hargett N A & Podos S M (1978) The effect of 1.5% ... intraocular pressure *Acta Ophthal (Kbh)* 56 ...
Vale J & Phillips C I (1973) Practolol (Fraldin) eye drops as an ocular h... agent *Br J Ophthal* 57 210
Wright P (1975) Untoward effects associated with practolol adm... mucocutaneous syndrome *Br Med J* 1 595
Zimmerman T J & Kaufman H F (1977) Timolol A new Beta adre... agent for the treatment of glaucoma *Arch Ophthal* 95 601

Author s address
Niels Vesti Nielsen M D
Department of Ophthalmology
Central Hospital
4800 Nykobing Falster
Denmark

Department of Ophthalmology (Head Henrik Forsius)
University of Oulu Oulu Finland

THE LONG TERM HYPOTENSIVE EFFECT OF TIMOLOL MALEATE COMPARED WITH THE EFFECT OF PILOCARPINE IN SIMPLE AND CAPSULAR GLAUCOMA

BY

P J AIRAKSINEN

The long term intraocular pressure (IOP) lowering effect of a β adrenergic blocking agent timolol maleate in topical administration was compared with the effect of pilocarpine on simple and capsular glaucoma by means of diurnal pressure curves during a six month follow up

In simple glaucoma timolol was more effective than pilocarpine in lowering IOP In the follow up a significant but not marked increase of the IOP was observed In capsular glaucoma timolol was not effective enough but when it was co administered with miotics the IOP lowering effect was better than with either substance alone

Timolol induced no accomodative myopia miosis reduction of tear flow or other side effects It increased the outflow facility in simple glaucoma but not in capsular glaucoma During the trial the anterior chamber depth increased while the corneal thickness remained unchanged

Four out of the six eyes included in a previous report of secondary glaucoma due to chronic uveitis are still after one year of therapy controlled with timolol

Key words Anterior chamber depth – β adrenergic blockers – ciliary body – corneal thickness – glaucoma – optic disc perfusion – outflow facility – timolol

Timolol maleate is a specific β adrenergic blocking agent inhibiting both β1 and β receptors without intrinsic sympathomimetic or local anaesthetic activity (Hall et al 1975) As an ophthalmic solution timolol had an IOP

Received October 9 1978

lowering effect of 50 % in single dose study (Zimmerman & Ka[illegible] Kriegelstein 1978) In ocular hypertensives and open angle gla[illegible] IOP lowering effect with no serious side effects was observed (Ri[illegible] Boger et al 1978a) After ten weeks of maintenance therapy the eff[illegible] present (Boger et al 1978b Nielsen 1978)

The purpose of this study is as a clinical trial to compare t[illegible] hypotensive effect of timolol with the effect of pilocarpine in the t[illegible] simple and capsular glaucoma The patients served as their own c[illegible] effects of timolol on corneal thickness anterior chamber dep h o[illegible] pupillary diameter tear secretion blood pressure and pulse ra[illegible] followed

Material and Methods

The clinical material consisted of 15 patients (6 females 9 males[illegible] 72 years (mean 58 7) who all had elevated IOP with glaucomatous c[illegible] the optic disc and/or glaucomatous central visual field defec[illegible] study 10 patients (18 eyes) had been treated for simple glaucoma [illegible] pilocarpine for 3 to 152 months (mean 54) Five patients (8 eyes) w[illegible] glaucoma had received similar therapy for 12 to 28 months (mea[illegible] some cases adrenaline or acetazolamide had been added to the therap[illegible] been stopped prior to this study becuse of local and systemic [illegible] fistulizing operation had been performed on four simple and one c[illegible] glaucoma eyes Three of the fistulas had become obliterated and the [illegible] treated with pilocarpine One operated simple and one capsular gla[illegible] showed normal IOP and did not take part in the trial The pa[illegible] cluded in this study becau e they either had an uncontrolled IOP ([illegible] were inconveniently managed with miotics (5 eyes) or both (11 eyes

The patients had no history of chronic obstructive pulmonary d[illegible] other disorders contraindicating the administration of β adren[illegible] agents An informed consent was obtained from the patien[illegible]

The timolol ophthalmic solution used in this study cons[illegible] (0 25 %) or 5 mg (0 5 %) timolol per ml Benzalkonium chlor[illegible] added as a preservative Administered once daily timolol was dropped [illegible] hours twice daily at 9 00 and 21 00 hours

Diurnal pressure curves were recorded with patients recei[illegible] miotics (I) and when no glaucoma therapy was given (II) These [illegible] compared with diurnal pressure curves recorded with op[illegible] tion at the beginning (III) and at the end (I[illegible] f the s x m[illegible]

ocular pressures were measured daily with a standard Schiotz tonometer 30 10 00 14 00 and 18 00 hours Each diurnal pressure curve was recorded wo to nine days (mean 3 6) Additionally applanation tonometry was ormed twice daily On the initial visit the patients underwent a careful cal examination itemized from 1 to 12 in Table I The examinations listed 7 were carried out on the patients when they were untreated and again n on timolol on the final visit examinations 1 to 10 were made Addi ally the patients were seen two six and twelve weeks into the follow up od and examinations one through four in Table I were carried out If the was uncontrolled timolol was first added to the maximum of 0 5 % timolol e daily and then pilocarpine 2 % three to four times daily The ophthal copic appearance of the optic discs was compared with the s ereophoto hs Blod pressure and pulse rate were recorded daily On each visit the ents were questioned about subjective local and systemic side effects he statistical significance of the responses to different treatments was ulated by dependent t statistics

Table I
Examinations carried out during the trial

Slit lamp examination	
Applanation pressure of the eye	
Blood pressure and pulse rate	
Ophthalmoscopy	
Corneal thickness and anterior chamber depth	Haag Streit Pachymeter I II
Outflow facility	Electronic Schiotz tonographer Berkeley
Pupillary diameter	Goldmann perimeter
Central visual field	Friedmann analyser
Peripheral visual field	Goldmann perimeter
Schirmer test	
Gonioscopy	
Stereophotography of the optic disc	Allen stereo separator mounted on a Zeiss fundus camera

Results

The mean intraocular pressures of the diurnal pressure curves (M... the trial are presented in Fig 1 In simple glaucoma timolol ... in all eyes however in two patien s (3 eyes) the reduction was ... at the beginning of the trial but with additional pilocarpine therapy ... was maintained at a therapeutic level throughout the study In s... MIOP (III) at the beginning of the follow up was 9 6 mmHg or ... than MIOP (II) without therapy ($P < 0\ 001$) and 3 6 mmH... MIOP (I) with miotics ($P < 0\ 001$) During the follow up MIO... in 12 eyes and the mean change was 1 9 mmHg ($P = 0\ 01$) The final ... was 7 7 mgHg or 27 % below MIOP (II) without therapy ($P < 0$... significantly lower than MIOP (I) with miotics ($P = 0\ 05$) In ... glaucoma patient (2 eyes) the MIOP was considered too high ... control (od 28 5 os 23 75) and after the trial pilocarpine was a... therapy with subsequent decrease of MIOP to a therapeutic level (15 ... respectively)

In capsular glaucoma MIOP (III) at the beginning of th... 10 5 mmHg or 36 % lower than MIOP (II) without therapy ($P <$... 6 3 mmHg or 25 % below the miotic MIOP (I) ($P = 0\ 004$) During th... up an increase of IOP was recorded in three eyes and pilocarpine was ... to the therapy The final MIOP (IV) at six months was 7.5 mmHg ...

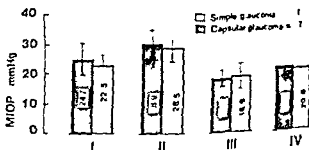


Fig 1

Mean intraocular pressure of diurnal pressure curve (MIOP) in simple ... glaucoma

MIOP with miotics (I) and without glaucoma therapy (II) compared w... beginning of the follow up with optimal timolol medication (III) and a... the six m nth follow up (IV) In simple glau oma during the f... capsular glaucoma at the end of the follow up three eyes were treated w... together with pil arpine

Vertical lines indicate stan ard deviat n

Table II

ity of outflow (μl/mmHg/min) in simple and capsular glaucoma In simple oma facility of outflow improved in 13 eyes out of 17 and showed no change in capsular glaucoma

		I	II	III	IV
le glaucoma	mean	0 126	0 110	0 142)	0 140)
	SD	0 047	0 056	0 061	0 074
I $P = 0 1$		II vs III $P = 0 01$		II vs IV $P = 0 008$	
IV $P = 0$?					
ılar glaucoma	mean	0 081	0 060	0 08	0 081*)
	SD	0 020	0 026	0 031	0 025
I $P = 0 03$		II vs III $P = 0 09$		II vs IV $P = 0$?	

n three eyes miotics were co administered
or key to Roman numerals see legend for Fig 1

w MIOP (II) without therapy ($P = 0 02$) and 3 3 mmHg or 13 4 % lower MIOP (I) with miotics ($P = 0 2$) The final recordings showed an un trolled IOP of another three capsular glaucoma eyes With additional carpine also to these eyes the MIOP was 5 5 mmHg or 22 % below MIOP with miotics ($P = 0 04$)

he values of outflow facility (Table II) were below normal throughout the ly in all eyes In simple glaucoma timolol induced a statistically signi nt increase of outflow facility At six months the effect was still present l was not different from the outflow facility with miotics In capsular glau a timolol unlike pilocarpine produced a nonsignificant increase of outflow ility

At the beginning of the study after miotics were stopped the pupillary dia ters were normalized and they were not affected by the subsequent timolol rapy The pulse rate (Fig 2 top) and the mean arterial blood pressure g ? bottom) were initially lowered At policlinic controls however the dings were no longer significantly different from initial values At the six nths control the pulse rate was again lowered after three days on the ward t the mean arterial blood pressure showed no significant change

The Schirmer test was made at the first and at the last visit. The tear flow

aqueous humor This is in agreement with earlier studies (Z[illegible] 1977 Kriegelstein 1978 Sonntag et al 1978)

The pulse rate was lowered at the beginning and at the end [illegible] when the patients were examined as in patients for a few days [illegible] may not be due to systemic absorption of timolol since the [illegible] small ca 0 5 mg daily at the most and because no significa[illegible] pulse rate was measured when the patients were examined as [illegible] more likely cause is the few days rest in the clinic and the [illegible] ment and anxiety often experienced by patients while at hospi[illegible] reduction of systemic blood pressure was detected with [illegible] diminished perfusion pressure of the optic disc

Some β adrenergic blocking agents have local side effects [illegible] local anaesthetic properties (Kriegelstein et al 1977) metoprol[illegible] allergic reactions (Ros et al 1978) oxprenolol produced corneal [illegible] (Holt & Waddington 1975) Systemic practolol induced oculo[illegible] syndrome (Wright 1975) the pathogenesis of which is possibly of [illegible] origin (Rahi et al 1976) Mackie et al (1977) reported that the [illegible] concentration of practolol patients was very low suggesting an [illegible] tion of the lacrimal gland They tested also patients receivin[illegible] the values were found either normal or somewhat increased In th[illegible] study no allergic reactions or decrease of the tear flow was [illegible] corneal thickness remained unchanged and no subjective or [illegible] of dry eyes were observed

Some of the patients in this study expressed a need for a [illegible] correction This as well as the increase of the anterior chamber [illegible] to be due in part to a gradual loss of the pilocarpine in[illegible] myopia

The ACD however increased significantly also during the [illegible] eyes which were treated only with timolol van Alphen (19[illegible]) [illegible] human ciliary muscle carries mainly β receptors and that the relax[illegible] catecholamines in eserine precontracted ciliary muscle strips can be [illegible] by propranolol Therefore the reason for ACD increase during [illegible] obvious and needs further investigation

Acknowledgment

I am grateful to Pekka Lankinen M Sc from th[illegible] Department of [illegible] of Oulu for his help in the statistical analysis of the material

This study was supported in part by the Medical Research [illegible]

References

Alphen G W H M (1976) The adrenergıc r ceptors of the ıntraocular muscles the human eye *Invest Ophthal 15* 50?–50?

?off P (1978) Erfahrungen mıt Tımolol ın der Glaukomtherapıe *Klın Mbl ?ugenheılk 173* 20?–207

r III W P Pulıafıto C A Steınert R F & Langston D P (1978a) Long term ?ence wıth tımolol ophthalmıc solutıon ın patıents wıth open angle glaucoma *phthalmology 85* 259–267

er III W P Steınert R F Pulıafıto C A & Pavan Langston D (1978b) Clınıcal ıal comparıng tımolol ophthalmıc solutıon to pılocarpıne ın open angle glaucoma *mer J Ophthal 86* 8–18

thene A (1976) The treatment of Glaucoma wıth propranolol (Inderal) A clınıcal ıal *Acta ophthal (Kbh) 54* 291–300

I R A Robson R D & Share N N (1975) Tımolol maleate A new beta adrenergıc ceptor blockıng agent *Arch Int Pharmacodyn 213* ?51–?63

t P J A & Waddıngton E (1975) Short report Oculacutaneous reactıon to xprenolol *Brıt Med J 2* 539–540

ty E & Hørven I (1978) Glaucoma treatment wıth tımolol *Acta ophthal (Kbh) 56* 0?–714

egelsteın G K Sold Darseff J & Leydhecker W (197?) The ıntraocular pressure esponse of glaucomatous eyes to topıcally applıed Bupranolol *Albrecht v Graefes Arch Ophthal ?0?* 81–86

egelsteın G K (1978) Dıe Wırkung von Tımolol Augentropfen auf den Augen ınnendruck beı Glaucoma sımplex *Klın Mbl Augenheılk 1?? 677–685

ckıe I A Seal D V & Descod J M (1977) Beta adrenergıc receptor blockıng drugs tear lysozyme and ımmunologıcal screenıng for adverse reactıon *Brıt J Ophthal 61* 354–359

elsen N V (1978) Tımolol hypotensıve effect used alone and ın combınatıon for treatment of ıncreased ıntraocular pressure *Acta ophthal (Kbh) 56* 504–509

dıus R L Dıamond G R Pollack I P & Langham M E (1978) Tımolol a new drug for management of chronıc sımple glaucoma *Arch Ophthal (Chıcago) 96* 1003–1008

hı A H S Chapman C M Garner A & Wrıght P (1976) Pathology of Practolol ınduced ocular toxıcıty *Brıt J Ophthal 60* 31?–3?3

tch R Hargett N A & Podos S M (1978) The effect of 1 5 % tımolol maleate on ıntraocular pressure *Acta ophthal (Kbh) 56* 6–10

?s F E Dake C L Nagelkerke N J D & Greve E L (1978) Metoprolol eyedrops ın the treatment of glaucoma a double blınd sıngle dose trıal of a beta adrenergıc blockıng drug *Albrecht v Graefes Arch Ophthal ?06* 247–?54

?arı K M Aıraksınen P J & Jaamo E A T (1978) Hypotensıve effect of tımolol on secondary glaucoma ın chronıc uveıtıs *Lancet I (8061)* 44?

onntag J R Brındley G O & Shıelds M B (19 8) Effect of Tımolol therapy on outflow facılıty *Invest Ophthal 17* 293–?96

Wrıght P (1975) Untoward effects assocıated wıth practolol admınıstratıon Oculo mucocutaneous syndrome *Brıt med J I* 595–598

Zimmermann T J & Kaufman H E (197?) Timolol a beta adrenergic ... for the treatment of glaucoma. *Arch Ophthal (Chicago)* 9? ...
Zimmermann T J Harbin R Pett M & Kaufman H E (19) T ... of outflow *Invest Ophthal* 1? 623-624

Author's address
P J Airaksinen M D
Department of Ophthalmology
University of Oulu
Kajaanintie 50
SF 90220 Oulu 22
Finland

Institute of Anatomy B University of Aarhus
(Former Head Th Blackstad)
and Department of Ophthalmology Aarhus Kommunehospital
(Head Niels Ehlers)

ANT-EGG CATARACT

An Electron Microscopic Study

BY

HENRIK DAA SCHRØDER and STEEN HOLST NISSEN

The ultrastructure of the ant egg cataractous lens has been studied Comparison of tissue demineralized by means of EDTA with untreated tissue showed the calcium salts in the ant eggs to be mostly crystalline

A laminar appearance of the ant egg seen in EDTA treated material suggested an intermittent growth of the structure

In the ant eggs as well as in some areas separate from these membrane limited cytoplasmic bodies could be seen in many cases the membranes of which were partly joint and partly separated by an electron dense material

It is suggested that the calcifications seen as the ant eggs are secondary phenomena to an abnormal metabolism morphologically seen as membrane limited cytoplasmic bodies

Key words ant egg cataract – ultrastructure – calcification – metabolic disorder

ne ant egg cataract is a hereditary zonular cataract characterized by a eyish opaque zone containing numerous ant egg like bodies (Riise 1967 Nis n & Schrøder 1978) A recently published paper has dealt with the clinical ndings and the heredity of this type of cataract as well as the light micro opic appearance and the element content of the ant eggs (Nissen & Schrøder 978)

eceived October 18 19 8



Figs 1 and 2

1 Transition zone between an ant egg and the surrounding tis[illegible] of the micrograph many crystals (C) are to be seen as very dark [illegible] less calcified tissue membrane limited cytoplasmic bodies with (M) [illegible] membrane appendices are found [illegible]20 000

2 Transition zone after EDTA treatment The crystal seen in Fig 1 has [illegible] but empty spaces (examples at E) with a localization and shape [illegible] are seen instead The dense material (D) and the membranes (M) [illegible] treatment [illegible]20 000

ıe present study was undertaken ın order to further elucıdate the structure the ant egg In addıtıon a structural explanatıon of the opaqueness of the of the lens contaınıng the ant eggs was looked for

Materıal and Methods

rated tıssue from an ant egg cataract was fıxed ın cold 3 % glutaraldehyde buf d wıth 0 15 M phosphate buffer (pH 7 9) After 3 h of fıxatıon the tıssue was fıxed for one h ın 1 % OsO_4 dehydrated ın graded ethanol and embedded ın n 819 Crude aspırate as well as ısolated ant eggs were embedded Part of the erıal was demıneralızed for 5 days ın 10 % EDTA ın 0 1 M trıs buffer pH 7 3 at before postfıxatıon and embeddıng (Fullmer & Lınk 1964)

ltrathın sectıons were cut and staıned wıth uranyl acetate 30 mın and lead cıtrate ın

Results

ht mıcroscopıcally the ant egg has been descrıbed as consıstıng of a homo neous core surrounded by a zone of a wavy appearance at the transıtıon to parently normal tıssue (Nıssen & Schroder 1978)

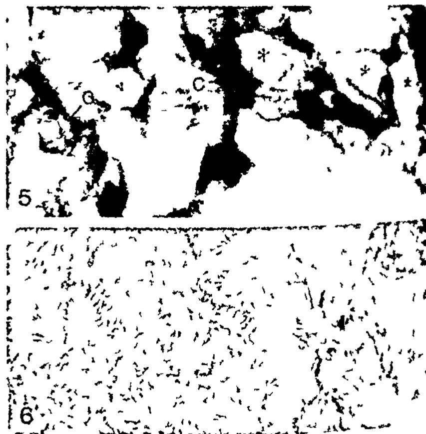


Fıgs 3 and 4

Crystals seen ın the transıtıon zone These lamınated structures dısappear when ubjected to EDTA treatment × 100 000

Hıgh magnıfıcatıon of a membrane lımıted cytoplasmıc body The fıve layers of he joınt membranes (arrows) as well as the swellıngs contaınıng dense materıal arrowheads) are seen × 100 000

The transition zone was at electron microscopic level cha... interdigitation of irregular relatively electron dense processes f... and more translucent processes from the surroundings (Fig ...). Between these two components a distinct dark layer of vari... was often found. At high magnifications this was seen to be la... Structures of a similar appearance could also be seen in the da... of the transition zone. In the EDTA treated material these la... had disappeared leaving empty spaces (Fig 2).



Figs 5 and 6

5 Ant egg core. The hardness of the calcified core results in a par... of the tissue during the sectioning with a consequent l... of material... Crystals (C) are however seen in the remaining tissue. 57 000

Core of the ant egg after treatment with EDTA. The crystals are ... empty needle shaped spaces smaller and more regular to the left th... 21 000

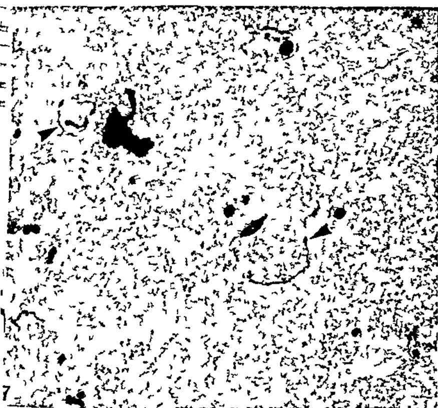


Fig 7

icrograph from a tissue block not containing ant eggs Several cytoplasmic bodies with associated membrane material are seen (examples at arrowheads) × ?0 000

In the periphery of the transition zone electron dense bodies surrounded by ı approximately 9 nm thick membrane were found (Fig 1) These bodies were ten continuous with some linear structures which at higher magnifications ›peared to be five layered with alternating light and dense lines (Fig 4) he central dense line was often more electron dense and thicker than the two :ripheral dense lines The five layered structure most likely represented a ısion of two adjacent membranes

The membrane limited cytoplasmic bodies resisted EDTA treatment (Fig 2) *The core* of the ant egg was not easily studied in mineral containing tissue nd only a few laminated structures were seen (Fig 5) When decalcified a ıcture very similar to that of the border zone was observed apart from a more eedle shaped appearance of the dissolved structures in the core (Fig 6) It

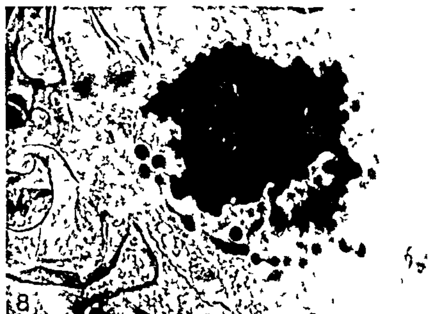


Fig 8
Membrane limited globules and processes most of which are ly... of fibre cytoplasm × 20 000

appeared as if the core could be separated into more concentric ... differed in respect to size and density of the needle shaped struct... Cytoplasmic bodies similar to those seen in the border zone were ... in the core

In some pieces of *tissue not containing ant eggs* areas with ... plasmic bodies and associated joint membranes were found Their ... was similar to that seen in the transition zone of the ant egg ... and shape was more variable (Fig ...)

In the present material no ribosomes were observed Some ... found and some electron dense material bearing similarities to ... as well as some with a less obvious origin (Fig 8) was seen

Discussion

The appearance of the laminated curved structure of the border zone ... with that of a crystalline structure The disappearance of the ... EDTA treatment indicates a calcium salt nature of the crystals ...

ement with the previous recordings of calcium and phosphorus in the ant (Nissen & Schroder 1978) The finding of crystal like structures in the core the similarity in effect of FDTA on the core and the transition zone of the egg resulting in needle shaped empty spaces indicate that also much of calcium and phosphorus of the core is present as crystals

he existence of more concentric shells in the core of the ant eggs as well he presence of a border zone would be in agreement with a progressive but rmittent growth of the ant egg It has however not been possible to obtain rmation on the development of the ant egg cataract in the previously lished papers

he localization of the electron dense bodies with appendices in the vicinity he ant egg as well as in tissue blocks not containing ant eggs suggests that e structures may be the structural background of the opaqueness found out the ant eggs However due to the mixture of lens material during aspira , it is impossible to prove that the dense body containing material originates n the periphery of the lens

he fusion of two adjacent membranes has previously been described in the athyroid hormone stimulated osteoclast (Lucht & Maunsbach 1973) Here y were found to be a part of the endoplasmic reticulum and they were taken evidence for an increased synthesis of membranes In the present study vever the deficiency of ribosomes means that no significant protein syn sis is taking place It is however a possibility that the complexes are part the smooth endoplasmic reticulum This organelle is known to be involved the synthesis of lipids and polysaccarides which could account for the pre ce of electron dense material within the structures

Another possible interpretation is that the cytoplasmic bodies could be more less collapsed lysosomes They could then be involved in an autophagocyto Also the possibility that the cytoplasmic bodies are secretion vacuoles has be mentioned

None of the mentioned organelles are seen in normal lens fibres (Cohen 13 Rafferty & Goossens 1977) The presence of the cytoplasmic bodies there e indicates that the normal metabolism of the lens fibres is disturbed

In other studies on lens fibre ultrastructure in cataracts an increased amount membrane material appeared (Dilley et al 1976) and sense bodies or large as with an appearance similar to that seen in the present Fig 8 were also served However in none of these studies could membrane limited cyto smic bodies similar to those characteristic for the ant egg cataract be found In other types of cataracts including congenital cataracts an altered meta lism may lead to necrosis and precipitation of various compounds e g cal im salts (Duke Elder 1969) Thus the calcium containing ant eggs which

clinically characterize the ant egg cataract might well be th... but unspecific calcium deposition due to an altered met... fibres morphologically observed as cytoplasmic dense bodies

Acknowledgment

We are greatly indebted to Ilona Bach Sørensen Bjarne Krunder... mann for their skilful technical assistance

References

Cohen E I (196-) The electron microscopy of the normal human lens ... 4 433–446

Dilley K J Bron A J & Habgood J O (19-6) Anterior pol... capsular cataract in a patient with retinitis pigmentosa ... ultrastructural study *Exp Eye Res 22* 155–167

Duke Elder S (1969) *System of Ophthalmology* Vol 11 pp ... Kimpton London

Fullmer H M & Link C C (1964) A demineralization procedure f... chemical use A quantitative succine dehydrogenase assay *S...* 35 -396

Lucht U & Maunsbach A B (19-3) Effects of parathyroid horm... in vivo An ultrastructural and histochemical study *Z Zellfo...*

Nissen S H & Schrøder H D (19-9) Ant egg cataract ... dominantly inherited congenital (ant egg) cataract including a ... tion of the formed elements *Acta ophthal (Kbh)* 5- 14 19

Rafferty N S & Coossens W (19) Ultrastructure of trauma... the frog A comparison with mouse and human lens *Amer J* ...

Riise R (196) Hereditary ant egg cataract *Acta ophthal (Kbh)* ...

Author's address
Henrik Daa Schrøder
Institute of Anatomy B
University of Aarhus
DK 8000 Aarhus C
Denmark

Central Hospital of Paijat Hame
(Head of the Eye Department P Pohjanpelto)
Lahti Finland

ANTERIOR CHAMBER HAEMORRHAGE IN THE NEWBORN AFTER SPONTANEOUS DELIVERY

A Case Report

BY

PEKKA POHJANPELTO KYRÖ NIEMI and TIMO SARMELA

A case report is presented of anterior chamber haemorrhage occurring in one eye in a newborn after spontaneous delivery At the age of two weeks the anterior chamber was clear but the vitreous cloudy At the age of five weeks the vitreous had also cleared The infant s later development was normal and there were no disorders in the function of the eye

Key words birth injury – intraocular haemorrhage in the newborn – hyphaema

...inal haemorrhages in the newborn are common The reported incidences ...y from 2 6 to 50 per cent (Chace et al 1950 Kauffman 1958 Krauer ...yer 1965 Neuweiler & Onwudiwe 1966) The big variation seems to be ...e to case selection and time of examination Owing to the rapid absorption retinal haemorrhages are most commonly noted shortly after birth ...emorrhages into the vitreous are considerably more infrequent Wiznia & ...ice (1976) collected in addition to their own two patients 10 reports de...ibing a total of 18 cases from the earlier literature

Anterior chamber haemorrhage in the newborn is an exceptional birth ...uma The rare cases reported in the literature involved forceps delivery ...ere the haemorrhage into the anterior chamber was the consequence of ...vere trauma caused by the forceps leading to the infant s death (Homer

...ceived October 16 1978

1884 Lomer 1884 Wintersteiner 1899 Thomson & P ... et al (1974) reported on a newborn with hyphaemas ... tion caused by the forceps The infant was diagnosed to have ... coagulation and died 36 h after birth

This is a report on a case in which anterior chamber ... established in a newborn after spontaneous delivery where ... cloudiness probably also blood was observed in the vitreous ...

The mother was a healthy primipara of 23 years ... in the 32nd week of pregnancy and occasionally an elevated ... recorded No treatment was given Labour began spontaneously ... pregnancy It lasted 8 h and 25 min the duration of the second ... 15 min The amniotic fluid was normal The baby a girl was d... cephalic presentation A coil of the umbilical cord emerged ... presenting part Delivery of the shoulders progressed with difficu...

The infant s birth weight was 3900 g length 50 cm and head circ... Her general condition was good apart from mild cyanosis The ... There was slight facial haematoma The left arm was hypotonic ... to a fracture of the clavicle

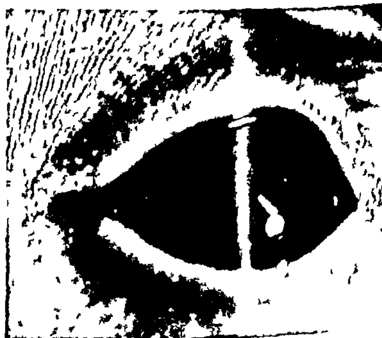


Fig 1
Infant s left anterior chamber full of bl... at the age of ...

xamination of the infant s eyes at the age of two days showed them to be ab ially red and an ophthalmologist was consulted
ne 3rd day of life Both conjunctivas revealed minor haemorrhages The left an r chamber was full of blood of coagulated appearance (Fig 1) The right ante chamber was completely clear macroscopically
t two weeks Both anterior chambers were clear on slit lamp examination. The vitreous was clouded and the red reflex absent The right vitreous was clear
t five weeks Both anterior chambers and vitreous bodies were clear
ollow up at 3½ years Vision of the right eye was 0 8 refraction +1 5 sph +0 5 cyl 30° there was some pigment on the surface of the lens remnants of a pupillary mbrane were not observed, the anterior chamber and vitreous were clear The thalmoscopy finding was normal The vision of the left eye was 0 8 refraction 5 sph there were slight remnants of a pupillary membrane and some pigment was ble on the surface of the lens the anterior chamber and vitreous were clear The il moved freely and the iris appeared normal without any abnormal transparency ophthalmoscopy finding was normal Gonioscopy o sin. chamber angle open ierous iris processes no recession of the angle no iris synechiae Gonioscopy of the t eye was not performed because of the co operation problems with a young child aediatric examination disclosed nothing abnormal compared with a healthy child. girl had been healthy throughout The mother had not detected anything indi ve of an increased bleeding tendency The blood values were considered to be nal. The thrombocyte count was 400 000 mm³ bleeding time was 2 min 30 sec and ulation time 150 sec

Discussion

o our knowledge not a single case of anterior chamber haemorrhage in the wborn after spontaneous delivery has been reported in the earlier literature en large series up to 10 000 patients in which retinal haemorrhages of the wborn were studied contain no mentions of hyphaema which would be very y to diagnose as a subsidiary finding (Chace et al 1950 Kauffman 1958 uer Mayer 1965 Neuweiler & Onwudiwe 1966) Birth traumas in the pre usly reported cases of anterior chamber haemorrhage in the newborn were severe that the patients died The child described here was not found to e any other injuries except for the fracture of the clavicle and her de lopment was normal The blood had disappeared from the anterior chamber the age of two weeks and the vitreous had cleared three weeks later The reous opacity was probably also due to haemorrhage although its absorp n generally takes several months in a newborn (Braendstrup 1969)
Retinal haemorrhages of the newborn have been attributed to congestion of retinal veins during delivery This aetiology does not seem to be correct regards anterior chamber haemorrhage The causative agent is probably a al trauma for hyphaema of the newborn is extremely rare and in e g

the case now reported it was unilateral The causative agent ... viously described has been a mechanical lesion caused by f... exception is possible the patient described by Azar et al ... found to have haemorrhages associated with disseminated ... lation in different organs A possible causative agent in th... the umbilical cord which was forced out simultaneously with ...

References

Azar P., Smith R S & Greenberg M H (1974) Ocular findings in d... vascular coagulation *Amer J Ophthal 78* 493–496

Braendstrup P (1969) Vitreous haemorrhage in the newborn. A ... intraocular haemorrhage *Acta ophthal (Kbh) 47* 507 513

Chace R R Merritt K K & Bellows M (1950) Ocular findings in th... *Arch Ophthal (Chicago) 44* 236–242

Homer (1884) cited by Thomson & Buchanan (1903)

Kauffman M L (1958) Retinal hemorrhages in the newborn *Amer J ...* 658–660

Krauer Mayer B (1965) Retinahaemorrhagien beim Neugeborenen ... tersuchungen nach Spontangeburten Vakuumextraktionen und F... *(Basel) 201* 168–176

Lomer (1884) cited by Thomson & Buchanan (1903)

Neuweiler W & Onwudiwe E U (1966) Retinahamorrhagien beim N... *Gynaecologica 162* 308–310

Thomson F & Buchanan L. (1903) A clinical and pathological account of ... injuries to the eye of the child during labour *Trans ophth I S ...*

Wintersteiner H (1899) Beitrag zur Kenntnis der Geburtsverle... *Z Augenheilk 2* 443–454

Wiznia R A & Price J (1976) Vitreous hemorrhages and dissem... coagulation in the newborn *Amer J Ophthal* ...

Author's address

Pekka Pohjanpelto
Honkapirtinkatu
15300 Lahti 90
Finland

The Department of Ophthalmology (Head \ Ehlers)
Århus Kommunehospital University of Aarhus Denmark

FIBRINOLYSIS AND TRAUMATIC HYPHAEMA

BY

THORKILD BRAMSEN

Since January 1st 1975 310 patients with traumatic hyphaema have been treated with the antifibrinolytic drug tranexamic acid One secondary haemorrhage has occurred corresponding to a frequency of secondary haemorrhage of 0 32 % Eighty five of these patients were treated as out patients
In four patients with traumatic hyphaema who were not treated with tranexamic acid, the serum content of activator inhibitor was determined daily An increase was seen during the first five days after the trauma followed by a marked fall on the 6th day In the same four patients the central corneal thickness was followed by daily measurements and compared to the variation in activator inhibitor

Key words traumatic hyphaema – fibrinolysis – tranexamic acid – activator inhibitor – central corneal thickness

has been previously described (Bramsen 1976 1977) the antifibrinolytic g tranexamic acid (Cyklokapron®) has been used in the treatment of umatic hyphaema on the supposition that secondary haemorrhages occur a fibrinolytic basis This supposition is supported by the favourable results ained in the previous studies (secondary haemorrhage frequency 0 68 %) e time of onset of the secondary haemorrhages also indicates a fibrinolytic gin. Moreover *Pandolfi (1978) has stated that high concentrations of fibrin* radation products (FDP) are found in the aqueous humour of patients with ondary haemorrhages This demonstrates that fibrinolysis occurs in the erior chamber in these patients Pandolfi & Kwaan (1967) also found that s tissue and to a lesser extent cornea endothelium was rich in plasminogen ivators For ethical reasons it is not possible to study the fibrinolytic

ceived January 4 1979

activity in the aqueous humor in humans at different tim[illegible] a traumatic hyphaema However the possibility that trauma [illegible] affect the fibrinolytic system of the whole organism has [illegible] Ygge (1972) studied the changes in concentration in the serum of fibrinolytic factors among others the factor activator [illegible] abdominal operations He found that the activity of this fa[illegible] was markedly increased during the first 5-6 days only to fa[illegible] around the 6th to 7th day It is understandable that abd[illegible] influence the fibrinolytic activity of the whole organism a[illegible] to examine whether trauma to the eye is likewise accompan[illegible] the serum concentration of activator inhibitor

Knight et al (1977) showed that changes in fibrin lysis [illegible] depended upon the *individual response which differed from p[illegible]* and did not depend upon the extent of the surgical trauma [illegible] sible to examine the changes in the content of the aqueo[illegible] patients it was instead decided to examine the changes in the se[illegible] by taking daily blood samples It was essential that the pa[illegible] part in this study did not receive any form of antifibrinolytic t[illegible]

In double blind trial by Bramsen et al (1978) it was shown th[illegible] acid had a reducing effect on cornea oedema and a possib[illegible] between the fibrinolytic system and central corneal thickness wa[illegible] In those patients where the serum content of activator inhib[illegible] determined measurements of central corneal thickness were a[illegible] order to determine whether there was any connection between t[illegible] in these two parameters

Tranexamic acid is a potent antifibrinolytic drug Fibrino[illegible] during bodily exercise (Biggs et al 1947) but can be effectively [illegible] this drug From the assumption that secondary haemorrhage occurs [illegible] lytic basis it should therefore be unnecessary to immobilize pa[illegible] traumatic hyphaema when treated with an antifibrinolytic agen[illegible]

In a study by Bramsen (1977) 75 patients with traumatic hyphaema [illegible] treated with tranexamic acid The patients were mobile [illegible] *no case of secondary haemorrhage occurred* In the presen[illegible] in patients treated on an out patient basis are presented

Material

The examinati[illegible] place between 1 1 19[illegible] 1/10-19[illegible] From [illegible] the material c[illegible] patients referred to the depar[illegible] haema Only th[illegible] patients with macroscopic hyphaema were [illegible]

78 patients consisted of 64 males (average age 17 5 years with a range from 5– ears) and 14 females (average age 23 2 years with a range from 7 to 45 years) other eye lesions apart from hyphaema are shown in Table I
om 1/1–1/10 1978 the material comprised 85 patients with traumatic hyphaema during this period only those patients with macroscopically visible hyphaema included The patients consisted of 63 males (average age 24 1 years with a e from 3 to 71 years) and 22 females (average age 23 6 years with a range from 77 years) The eye lesions in addition to the hyphaema are shown in Table I
ur male patients with macroscopically visible hyphaema took part in the daily minations of activator inhibitor content in the serum and measurements of central al thickness The ages of these patients were 27 22 24 and 54 years respectively eye lesions apart from hyphaema are shown in Table I

Methods

patients with the exception of the four who had the serum content of activator ibitor measured were treated with oral tranexamic acid 25 mg/kg/day three times ly This was the sole form of treatment and started on the day of referral From period 1/1 1977 until 31/12 1977 the patients were admitted to the ward for a iod of five days but they were mobile without any eye bandaging There were no tnctions in the patients physical activity From the period 1/1 1978 until 1/10 1978 patients were admitted to the ward on the day of referral and instructed in the

Table I

	Period 1/1 77 until 31/12 77	Period 1/1 78 until 1/10 78	Four patients treated with bed rest
Subconjunctival haematoma	8	12	1
Corneal erosion	26	24	2
Pupillary changes	46	41	3
Iridodialysis	3	4	
Increased intraocular tension	6	10	1
Traumatic cataract	2	4	
Retinal haemorrhage	4	7	
Central retinal oedema	6	8	
Choroidal rupture	3	2	
No associated lesions	*12*	*14*	–
No	78	85	4

esions associated with traumatic hyphaema

proper administration of tablets The patients from this period were dis[illegible] the following day and allowed to resume their normal work and activ[illegible] 0-6 day following the trauma all the patients were re-examined includ[illegible] examination, ophthalmoscopy tonometry and visual acuity [illegible] formed in patients over 10 years of age. Twelve patients did not [illegible] examination.

The four patients who did not receive the antifibrinolytic treatment [illegible] the time of admission, treated with bed rest and stenopaeic glasses This [illegible] continued for six days At the same time daily measurements of [illegible] thickness were performed, and venepuncture was carried out to determ[illegible] inhibitor content. The estimation of this factor was carried out in the fo[illegible] The venous blood was kept in a plastic container at −60 C. The analysis of [illegible] inhibitor was carried out using the method described by Paraskevas et al. [illegible] pool of venous blood from 40 healthy individuals was used as a [illegible] for the examination. A standard curve was drawn from a dilution [illegible] this pool (1 1 1.5 1 10 1 20 and 1 40) The percentage concentra[illegible] inhibitor content in the patients serum was determined from this curve. Th[illegible] of the method is as follows – fibrinogen, trombin, plasminogen and [illegible] added to the patients serum. The fibrinogen is changed by the thrombin [illegible] fibrin clot. The urokinase activates plasminogen into plasmin. The plasm[illegible] the fibrin which then becomes no longer visible. The time interval [illegible] clot is dissolved depends upon the serum content of inhibitor

Results

No secondary haemorrhages occurred among the 78 patients who wer[illegible] ted to the ward without being confined to bed during the period [illegible] until 31/12 1977 Examination on the 12th day revealed visual [illegible] gonioscopic findings as shown in Table II Neither were there any [illegible] haemorrhages among the 85 patients treated as out patients in [illegible] period 1978 until 1/10 1978 in spite of the fact that almost all the patients [illegible] their normal working activity The visual acuities and gonioscop[illegible] on the 12th days are shown in Table III

In the four patients treated with bed rest, without antifibrinolytic [illegible] there were no secondary haemorrhages Fig 1 shows the average curve [illegible] variations in the serum content of activator inhibitor ($\bar{x} \pm$ SEM) [illegible] average curve of the variations in the central corneal thickness ($\bar{x} \pm$ [illegible] As can be seen from the curve of the activator inhibitor content a [illegible] occurred during the first two days (concentration about 80 % of [illegible] Following this the inhibitor in the serum increased and reached a [illegible] on the 5th day (165 %) Between the 5th and 6th day a fall [illegible] from 165 % to around the normal of 100 %

Table II

Visual acuity		Gonioscopy	
1 0	46	Normal	36
0.5–0 9	18	Goniosynechias	4
0 3–0 4	3	Traumatic angle	10
0 1–0.3	2		No
0 1	1		No

The visual acuities and gonioscopic changes on the 12th day following traumatic hyphaema in 8 in patients treated with tranexamic acid for 6 days following the trauma Eight patients did not show up for re examination

The average curve of the central corneal thickness showed an unchanged dema for the first two days This was followed by a fall during the period tween the 2th and 5th day (during which time the inhibitor in the serum creased) A secondary increase in thickness occurred between the 5th and h day to be followed by a renewed fall in thickness The secondary increase thickness occurred simultaneously with the marked fall in serum content of tivator inhibitor

Table III

Visual acuity		Gonioscopy	
1 0	62	Normal	52
0 5–0 9	13	Goniosynechias	5
0.3–0 4	5	Traumatic angle	3
0 1–0.3	3		
0 1	0		

The visual acuities and gonioscopic changes on the 12th day following traumatic hyphaema in 85 out patients treated with tranexamic acid for 6 days following the trauma Four patients did not show up for re examination

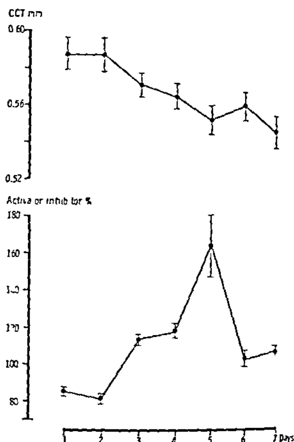


Fig 1
The average curve of the variations in the serum content of activator [illegible] the average curve of the variations in the central corneal thicknes ($\bar{x} \pm$ SEM) [illegible] patients with traumatic hyphaema



Fig 2
The variation in the serum content of activator inhibitor in a healthy member [illegible] staff in the department

Discussion

tifibrinolytic treatment is considered to be effective in preventing secondary morrhage following traumatic hyphaema In the course of 1975 72 patients h traumatic hyphaema were treated with bed rest stenopaeic glasses and nexamic acid and one case of secondary haemorrhage occurred (Bramsen 6) During 1976 75 mobilised patients were treated with tranexamic acid l no secondary haemorrhages occurred (Bramsen 1976) This paper present results of the antifibrinolytic treatment of "8 mobilised in patients and out patients There were no cases of secondary haemorrhages Altogether) patients with traumatic hyphaema have been treated with tranexamic d with one case of secondary haemorrhage corresponding to 0 32 % Of se only 72 patients have together with the antifibrinolytic treatment been ated with bed rest (the one case of secondary haemorrhage occurred in this up) The present material therefore suggests that restriction in physical ivity during antifibrinolytic activity is not relevant as far as the occurrence secondary haemorrhage is concerned

Studies on the changes in the central corneal thickness and the serum content activator inhibitor were only undertaken in four patients The results ggest that changes in both parameters occur following ocular trauma In e present study there was a close correlation between the changes in the ntral corneal thickness and the serum content of activator inhibitor in that e corneal thickness decreased while the inhibitor in the serum increased id secondary increase in corneal thickness occurred at the time of a fall the concentration of activator inhibitor Bramsen & Stenbjerg (1979) studied e same factor in the serum after operative intervention on the eye and found similar correlation to the changes in corneal thickness in the majority of ie patients

As described in the introduction various authors have studied the changes 1 the fibrinolytic activity in connection with operative interventions and milar results have been found As mentioned Knight et al (1977) found iat these changes were independant of the extent of the operative trauma hese authors examined the fibrinolytic response to trauma in 53 patients nd found that a fall in fibrinolytic activity from the day following operation ccurred in 81 % of patients whereas 19 % had a higher activity on the first ostoperative day than preoperatively and here the fall in activity during he following days was gradual and slight Secondary haemorrhages could ossibly occur in this last group

In the Nordic countries the frequency of secondary haemorrhages lies onsiderably below 19 % This could be due to the fact that the treatment

with bed rest is effective At the present time it is not possible to [illegible] patients who are capable of effectively inhibiting fibrinolysis after a [illegible] As secondary haemorrhages have such serious consequences it must be [illegible] able to treat all such patients with an antifibrinolytic agent.

The repeated venepunctures on the patients in whom [illegible] activator inhibitor were undertaken can be said in themselves to be [illegible] Daily blood samples for measurement of serum activator inhibitor [illegible] tion were taken from one of the members of the staff in the [illegible] order to determine whether the taking of blood samples could [illegible] changes The result is shown in Fig 2 The changes are quite [illegible]

References

Biggs R MacFarlane R. G & Pilling J (194[illegible]) Observations on fibrin[illegible] mental activity produced by exercise or adrenaline. *Lancet 1* 40[illegible]-40[illegible]

Bramsen T (19[illegible]6) Traumatic hyphaema treated with the antifibrinolytic [illegible] xamic acid. *Acta ophthal (Kbh)* [illegible]4 2[illegible]0-2[illegible]6

Bramsen T (19[illegible]7) Traumatic hyphaema treated with the antifibrinolytic [illegible] xamic acid II *Acta ophthal (Kbh)* 5[illegible] 616-620

Bramsen T. Corydon L. & Ehlers N (1978) A doubleblind study of the [illegible] tranexamic acid on the central corneal thickness after cataract [illegible] *ophthal (Kbh)* [illegible]6 121-126

Bramsen T & Stenbjerg S (1979) Variations in serum antifibrinolytic [illegible] lowing eye operations *Acta ophthal (Kbh)* in press

Knight M T N Dawson R & Melrose D G (19[illegible]) Labile and stable [illegible] their relavance to post operative deep venous thrombosis *Lancet* [illegible]

Pandolfi M (1978) Intraocular haemorrhages A hemostatic therape[illegible] *Surv Ophthal* 22 322-334

Pandolfi M & Kwaan H C (1967) Fibrinolysis in the anterior segment of [illegible] *Arch Ophthal (Chicago)* [illegible] 99-104

Ygge J (19[illegible]0) Changes in blood coagulation and fibrinolysis during the [illegible] period. *Amer J Surg 119* 22[illegible]-232

Author's address

Dr T Bramsen
Department of Ophthalmology
Århus Kommunehospital,
DK 8000 Århus
Denmark

The Department of Ophthalmology (Head N Ehlers)
Århus Kommunehospital University of Aarhus Denmark

SERUM AND AQUEOUS HUMOUR CONCENTRATION OF TRANEXAMIC ACID AFTER PERORAL ADMINISTRATION

BY

THORKILD BRAMSEN

After a single oral dose of 25 mg/kg tranexamic acid in 37 patients with cataract as the sole eye disease the concentration of the drug was measured in both the serum and the aqueous at various intervals following intake

The serum concentration was highest after three h (average 15 44 mg/l) but a trace of tranexamic acid (0 7 mg/l) could still be found after 19 h In the aqueous the concentration was likewise highest after three h (average 1 67 mg/l) but following this the fall in tranexamic acid concentration was very gradual and after 19 h was found to be 1.3 mg/l

In two patients who had received 25 mg/kg three times daily for three days an aqueous concentration of 2 3 mg l was found eight h after the final intake

Key words Aqueous – cataract – tranexamic acid – serum

n ophthalmology tranexamic acid has been used in the treatment of hyphaema Bramsen 1976 1977) and in the prevention of secondary haemorrhages fol wing cataract operations (Jerndal & Frisen 1976) The drug has also been own to be able to reduce cornea oedema in Fuchs endothelial dystrophy Bramsen & Ehlers 1977) and to reduce the corneal oedema occurring after ataract operations (Bramsen et al 1978) Moreover tranexamic acid has been sed in the treatment of choriodal melanomas (Bramsen 1978)

Previously there has only been indirect evidence in favour of the drug pas ing the blood aqueous barrier The effect of the drug on pathological condi

Received January 23 1979

tions in the anterior segment could be indirect e g via the fibrino[illegible] the complement system or the amino acid transport system. A [illegible] aqueous concentration after intake of tranexamic acid is therefore of [illegible] for the understanding of the mechanism of action of the drug Th[illegible] report in the literature of any studies in humans concerning this Camp[illegible] (1976) showed that tranexamic acid was able to penetrate the cornea [illegible] but apart from this the tranexamic acid concentration in the aqueous h[illegible] been studied

Davson (1956) has pointed out the similarities between the comp[illegible] formation of cerebrospinal fluid and aqueous Tovi & Thulin (19[illegible]) reported on the concentration of tranexamic acid in the serum and cereb[illegible] fluid following injections of 1000 mg every hour for eight days The ex[illegible] tion was carried out on four patients who were treated with tranexamic in order to prevent secondary haemorrhages after aneurysmal subarach[illegible] haemorrhages The examination showed that the concentration in the ce[illegible] *brospinal fluid gradually increased following repeated injections*, wherea[illegible] serum concentration was not affected by this A concentration of 0.3 mg/l [illegible] found in the cerebrospinal fluid 30 minutes after injection The concentr[illegible] after 8 days treatment was 4 5 mg/l At the same time measurements of [illegible] cerebrospinal fluid concentration of fibrin split products (FDP) which [illegible] sign of the breakdown of fibrin were also undertaken The concentrat[illegible] FDP had fallen after 30 min in spite of the low concentration of tra[illegible] acid at that time FDP was no longer found in the cerebrospinal flu[illegible] eight days

Tovi & Thulin (1972) have shown that tranexamic acid is capable of p[illegible] from blood to cerebrospinal fluid It is however to be noted that the pa[illegible] in the study had suffered subarachnoid haemorrhages and conseq[illegible] bloodbrain barrier was most probably no longer intact This could expla[illegible] the concentration was slightly increased In the following study the res[illegible] the investigations of tranexamic acid content in the aqueous of the [illegible] *reported It is considered that the blood aqueous barrier is* intact

Material

The material comprised 39 patients who had been admitted for senile cataract [illegible] tions The average age was 67.5 years with a range from 50 to 83 years. On [illegible] a routine ophthalmological examination was performed to exclude the p[illegible] additional eye disease Only those patients where senile cataract was the [illegible] disease were accepted for the study Serum creatinine was determined in all pa[illegible] and only those with normal values were accepted

Methods

-ty seven patients received oral tranexamic acid 25 mg/kg at various time intervals r to operation (from 45 min to 19 h) The cataract operation commenced under .bulbar anaesthesia with paracentesis of the anterior chamber and aspiration of rox 200 µl of aqueous In two patients the amount of aqueous aspirated was fficient for determination of the tranexamic acid content Venepuncture for deter ation of the serum content of tranexamic acid was performed as soon as possible re or after the anterior chamber paracentesis The aqueous and serum were imme edly frozen to −18 C and in the frozen state were sent to the research department Stockholm where gas chromatography analysis was performed as described by ssman & Stromberg (1975) Two patients were treated with oral tranexamic acid m kg three times daily for three days and paracentesis and venepuncture were formed eight h after the last dose

he 37 values for serum concentration and the 35 for aqueous concentration were wn up on a co ordinate system Owing to the quite considerable range an average e was determined in the following way The average of all the values between h were grouped as for one hour The average of the values between 2–4 h were en as corresponding to 3 h and so on up to and including 10 h for the serum ues and 6 h for the aqueous values

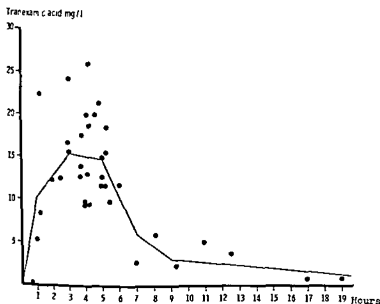


Fig 1

The serum concentrations of tranexamic acid after a single oral dose of 25 mg/kg measured at various intervals after intake. One point per person.

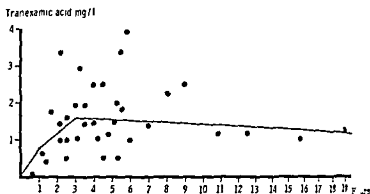


Fig 2
The aqueous humour concentrations of tranexamic acid after a single oral dose of 25 mg/kg measured at various intervals after intake One point per person

Results

Fig 1 shows the serum concentrations of tranexamic acid after a single oral dose of 25 mg/kg measured at various intervals after intake It can be seen that the average maximum concentration (15 44 mg/l) is reached after about 3 hours Following this there is a sharp fall between 3–9 h Tranexamic acid is however still found in the serum after 19 h

Fig 2 shows the aqueous concentrations of tranexamic acid following a single oral dose of 25 mg/kg measured at various intervals after intake The highest concentration (1 62 mg/l) is also found after about three h The graph however remains almost constant after this so that even after 19 h, the concentration approximates the 3–h value

The graphs also show that the aqueous concentration is about 10 % of the serum concentration for approximately the first five h After 17 h the aqueous concentration is higher than the serum concentration

In the two patients who had received tranexamic acid 25 mg kg three times daily for three days the serum concentrations were 3 6 mg/l and 8 4 mg/l respectively (average value 6 0 mg/l) eight h after the final intake This value is about 30 % higher than the average value found after a single oral dose The corresponding values for aqueous after eight h were 2 9 mg/l and 2.3 mg/l which is about 40 % higher than the average value after a single oral dose

Discussion

present study has shown that tranexamic acid capable penetrating the l aqueos barrier and that the concentration during the first five h after igle oral dose is approximately 10 % of the serum concentration Trane c acid is slowly eliminated from the anterior chamber of the eye More it is possible that as with cerebrospinal fluid accumulation occurs after ated intake of the drug In those cases where the blood aqueous barrier stroyed e g following ocular trauma and with inflammatory conditions anterior chamber it can be assumed that a larger concentration than nal can pass into the anterior chamber

ovi & Thulin (1972) found that a concentration of 0 3 mg/l was able to ice the content of FDP in the cerebrospinal fluid This suggests that even a low concentration has an inhibitory effect on the breakdown of fibrin i healthy eye with an intact blood aqueous barrier it can be assumed that r repeated intake with approxximately six h interval the concentration be higher than 0 3 mg/l It can therefore be assumed that the concentration ifficient to inhibit fibrinolysis

1 the case of traumatic hyphaema it is hardly the concentration of trane nic acid in the aqueous that determines the effectivity of the drug The nary clot is situated in the vessel wall and the activators of the fibrinolytic em are largely localised in the endothelium of the blood vessels It is there to be expected that it is the serum concentration that determines the effect he drug

Moreover the study suggests that there are quite considerable individual dif nces in the tranexamic acid serum concentration but less in the aqueous icentration after oral intake In a study on 12 healthy volunteer patients by ksson et al (in press) the serum tranexamic acid concentration was measured ? 3 4 5 6 and 9 h after oral and intravenous intake In this material ecially during the first four h a considerable individual difference in the icentration both after oral and intravenous administration was also noted ie pattern of the graph in the present study is similar to that found by Eriks 1 et al (1979) The range could be due to the uncertainty in determining the inexamic acid concentration The amount of aqueous or serum necessary for e measurement is about 150 µl (Veesman & Stromberg 1975) Analysis of mples in biophysiological studies have shown a variation coefficient of 1 4 % = 8) Samples with a very low concentration (about 235 mg/ml) had a varia on coefficient of 7 % (n = 8)

In the present study there is nothing inconsistent with a passive diffusion of anexamic acid across the blood aqueous barrier The higher concentration in


14
0.25μ
15
0.25μ

Results

ıorn human lens

ernated epithelial cells of the newborn human lens revealed intermediate ım) filaments as well as actin filaments (Fig 1) The actin filaments (5 nm ıeter) were mainly seen at the basal region of the epithelial cell close to epithelial fiber cell junction intermingled with 10 nm filaments Cortical nuclear fiber cells contained intermediate filaments and thin irregular fi 5 nm in diameter with attached particles (Fig 3) Negative stain analysis previously shown that the irregular fibrils referred to as chains consist of ıcles (15-20 nm diameter) arranged along a filament backbone of 5 nm ıeter (Bradley & Maisel 1978)

uclear fiber cells contained intermediate filaments however the chains were d both in single elongate forms as well as in aggregated configurations ?)

ıar-old human lens

anterior epithelial cells of the 24 year old lens contained 10nm filaments l actin filaments as observed in the newborn lens (Fig 4) Incubation of the ıerior epithelium with HMM exhibited arrowhead complexes on the actin work but not on the 10 nm filaments (Fig 5) Intermediate filaments nm) were present in the superficial cortical fiber cells (Fig 6) markedly uced in number in the deep cortical cells and absent from the nuclear fiber s Single elongated chains were abundant in the superficial cortical fiber s They appeared in aggregated and coiled form in the deeper cortical and lear fiber cells (Fig 7)

'ear-old human lens

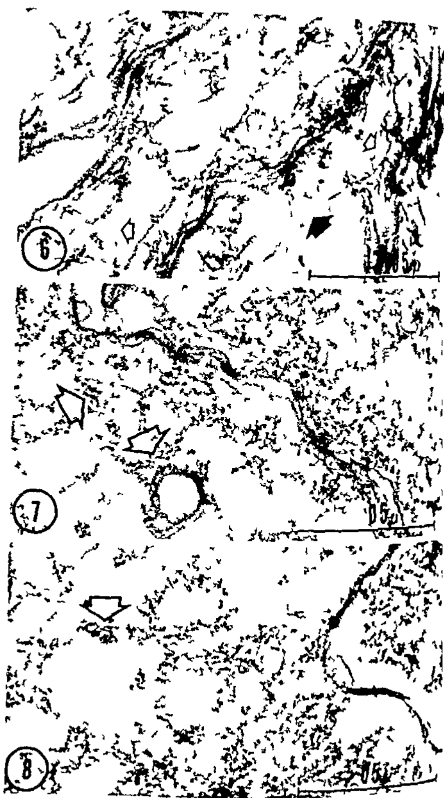
e 80 year old human anterior epithelium also contained many 10 nm fila nts as well as actin filaments No other area of the 80 year old lens de nstrated the presence of 10 nm filaments The protein chains in the cortex l nucleus were seen mostly in the aggregated form (Fig 8)

Fig 4

ycerinated epithelial cells of 24 year old lens showing 10 nm filaments (solid arrow ıds) and actin filaments (open arrowheads) The filaments were present on the epithelial side of the epithelial cell fiber cell junction 120 000 X

Fig 5

ubation of the epithelial cells with HMM demonstrates arrowhead complexes on actin filaments (open arrowhead) but no reaction on the 10 nm filaments (solid arrowheads) 120 000 X


6
7
8

Discussion

presence of cytoplasmic filaments in lens epithelial cells has previously n documented Actin has been identified by Longchampt et al (1976) in ine epithelial cells using immunofluorescence by Mousa & Trevithick (1977) he mouse epithelial cells and by Rafferty & Goossens (1978) in mouse frog grey tree squirrel and human lens epithelial cells Although microtubules e not seen in this study they have been reported in lens epithelial and erficial cortical fiber cells (Kuwabara 1975 Piatigorsky 1975 Bradley et al press) Microtubules are extremely sensitive to low temperature pH and ic *environment Under the conditions of this experiment micro ubules are* dily disrupted

their study of the unglycerinated human lens Rafferty & Goossens (1978) ed a heavy accumulation of filaments on either side of the epithelial fiber l junction especially on the cytoplasmic side of lens fibers abutting the over ıₒ epithelium In this study a heavy concentration of filaments (both actin l intermediate filaments) was noted on the epithelial side of the junction e absence of a fibrillar sheath on the fiber side of the junction in this study *y be due to the experimental conditions*

Although intermediate filaments were present in the epithelial cells in all ee ages their distribution in the fiber cells varied with age Thus they were ind in cortical and nuclear cells of the newborn lens only in the cortical er cell of the 24 year old lens and only in the epithelial cells in the 80 year l lens Even in the 24 year old lens the number of intermediate filaments reased markedly from the superficial to deep cortical fiber cells The ab ice of intermediate filaments from adult lens nuclear fiber cells is consistent h *previous studies on rat rabbit and chicken lens (Maisel 1977)*

The protein chains previously identified by negative stain as globules ar ;ged along a filamentous background appear in single elongated form in the erficial cortical cells In the deep cortical and nuclear cells they appear

---

Fig 6

/cerinated superficial cortical fiber cells of the 24 year old human lens contain nm filaments (solid arrowhead) and single elongate forms of chains of proteins *(open arrowhead)* 84 000 X

Fig 7

e nuclear region of the 24 year old lens contains only chains of protein in aggregated forms (open arrowhead) 84 000 X

Fig 8

ycerinated cortical fiber cells of the 80 year old human lens show aggregates of chains of protein (open arrowhead) but no 10 nm filaments 84 000 X

highly coiled and aggregated The chains were never identified in the ... cells Whether the backbone of the chains is actin remains to be ...

Although the number of lenses in this study is small the ... intermediate filaments are concentrated in the epithelial and superficial cells is consistent with previous observations (Maisel 1977 Bradley ... press) The role of the intermediate filaments in eukaryotic cells ... resolved A cytoskeletal function has been postulated as well as a role ... choring of the nucleus (Lehto et al 1978) Kuwabara (1975) pointed ... the lens appears to change its shape during accommodation mainly by ... cellular shape in the superficial fiber zone The relationship of the ... and actin filaments in this process requires further study Rafferty & ... (1978) suggested that the role of cytoplasmic filaments is either to ... support a spherical shape or to provide a contractile force during the ... of accommodation Whether loss of filaments is involved in the loss of ... modative ability with aging or in the cataractous process remains to be ... plored

Acknowledgment

This study is supported by PHS Research Grant EY 01417 from the ... Institute Bethesda Maryland USA

References

Bradley R H Ireland M & Maisel H The cytoskeleton of chick lens cells *Exp ... Res* (in press)

Bradley R & Maisel H (1978) Chain proteins of the vertebrate lens *Experientia* 4 0–4/1

Hazlett L & Bradley R H (1978) Spontaneous cataract in nude athymic ... structural analysis *Exp Eye Res 26* 207–217

Hynes R D & Destree A T (1978) 10 nm filaments in normal and transformed ... *Cell 13* 151–163

Ishikawa H Bischoff R & Holtzer H (1969) Formation of arrowhead ... with heavy meromyosin in a variety of cell types *J Cell Biol 43* 31 ...

Kuwabara T (1975) The maturation of the lens cell A morphological ... *Eye Res 20* 427–443

Lazarides E & Belzer D R Jr (1978) Specificity of desmin to avian and mammalian muscle cells *Cell 14* 429–438

Lehto V P Virtanen I & Kurki P (1978) Intermediate filaments anchor the ... in nuclear monolayers of cultured human fibroblasts *Nature (Lond)* ...

R Ransom L Kaufmann Y & Penman S (19..) A cytoskeletal structure with ociated polyribosomes obtained from HeLa cells *Cell 10* 67–78

champt M D Laurent M Courtois Y Trenschsv P & Hughes R S (1976) icrotubules and microfilaments of bovine lens epithelial cells Electron micro opy and immunofluorescence staining with specific antibodies *Exp Eye Res 23* 5–518

sel H (19.7) Filaments of the vertebrate lens *Experientia 33* 525–526

sel H Lieska N & Bradley R (1978) Isolation of filaments of the chick lens *xperientia 34* 35 –353

sel H & Perry M (1972) Electron microscopic observations on some structural oteins of the chick lens *Exp Eye Res 14* 7–12

isa G V & Trevithick J R (1977) Differentiation of rat lens epithelial cells in ssue culture II Effects of cytochalasins B and D on actin organization and ifferentiation *Dev Biol 60* 14–25

tigorsky J (1975) Lens cell elongation *in vitro* and microtubules *Ann N Y Acad ci* 253 333–347

lard T D Shelton E Weighing R R & Korn E D (1970) Ultrastructural naracterization of F actin isolated from *Acanthamoeba castellanii* and identifica on of cytoplasmic filaments as F actin by reaction with rabbit heavy meromyosin *molec Biol 50* 91–97

ferty N & Goossens W (1978) Cytoplasmic filaments in the crystallin lens of arious species Functional correlation *Exp Eye Res 6* 1 .–190

idich J A & Watt S (1971) The biochemical regulation of rabbit skeletal muscle ontraction *J biol Chem 246* 4866–4871

thor s address

Harry Maisel
partment of Anatomy
ayne State University
hool of Medicine
) E Canfield
troit Michigan 48201
S A

The Department of Ophthalmology (Head \ Ehlers)
and the Coagulation Laboratory (Head F Kissmeyer Nielsen)
Blood Bank Blood Grouping and Tissue Typing Laboratory
(Head F Kissmeyer Nielsen)
University of Aarhus Århus Kommunehospital Århus Denmark

FIBRINOLYTIC FACTORS IN AQUEOUS HUMOUR AND SERUM FROM PATIENTS WITH FUCHS DYSTROPHY AND PATIENTS WITH CATARACT

BY

THORKILD BRAMSEN and STENER STENBJERG

The concentration of α macroglobulin α_1 antitrypsin plasminogen C_3 complement fibrinogen degradation products (FDP) and fibrinolytic activity were studied in the aqueous humour and serum from nine patients with Fuchs endothelial dystrophy 17 patients with uncomplicated senile cataract and in the secondary aqueous from six cataract patients Finally the aqueous humour and serum from two patients anterior uveitis were studied

An increased concentration of α_2-macroglobulin α_1 antitrypsin plasminogen and C_3 complement was found in both the aqueous and the serum from patients with Fuchs dystrophy when compared with the primary aqueous and serum from patients with cataract but this was only significant for α_1 antitrypsin in aqueous humour A significant increase in the amount of FDP was found in the serum of the Fuchs patients compared with the cataract patients Fibrinolytic activity could not be demonstrated in the serum in any of the patient groups

The concentrations of the various factors found in the secondary aqueous of the cataract patients differed only slightly from the content of the primary aqueous of the Fuchs patients

Key words Fibrinolysis – tranexamic acid – Fuchs endothelial dystrophy – cataract – uveitis anterior – aqueous humour – complement

Received December 23 1978

nsen & Ehlers (1977) found that the antifibrinolytic agent tranexamic acid a reducing effect on the corneal oedema occurring in Fuchs dystrophy It therefore considered relevant to examine certain of the factors in the nolytic system in patients with this disease

orneal oedema o curs in connection with inflammatory conditions in the rior segment of the eye Measurable oedema is always present in acute rior uveitis (Mishima 1968) This oedema can occasionally be seen in the lamp as folds in Descemet s membrane The oedema which occurs on the is of an anterior uveitis can be assumed to be secondary to the inflammatory dition in the uvea

t was demonstrated in a doubleblind trial (Bramsen et al 9/8) that nexamic acid was able to reduce the corneal oedema occurring after cataract rations One possible explanation is that the secondary aqueous contains tors which either cause or maintain the oedema and that these factors ld be affected by an antifibrinolytic agent

On the basis of the supposition that the aqueous humour in patients with chs dystrophy in anterior uveitis and following operative intervention in anterior chamber contains factors which are responsible for the corneal dema it was decided to examine certain of the factors connected with the rinolytic system and complement system in the above mentioned groups of tients and in patients without corneal oedema

Material

he material consisted on nine patients with Fuchs endothelial dystrophy and ataract. The average age was 69 3 years with a range from 56 to 80 years A econd group consisted of 17 patients in which senile cataract was the sole eye isease In this group the average age was 73 8 years with a range from 53 to 94 ears Finally there were two patients with anterior uveitis both of whom had orneal oedema with normal intraocular tension The patients with Fuchs dystrophy nd the cataract patients were referred with regard to cataract operation The uveitis atients were seen because of recurrent inflammation complicated by cataract with isual impairment

Methods

All of the operations were performed under retrobulbar anaesthesia Prior to opera tion 250 µl of aqueous was aspirated Venepuncture was performed immediately after this Two aspirations were undertaken in six of the 17 cataract patients Approxi mately 10 min elapsed following removal of the primary aqueous until the anterior

chamber was reformed and in these six patients the reformed aqueous was [illegible] (approx 100 μl)

Serum and aqueous humour were applied without delay to the fibrin p[illegible] test material was frozen at −20°C for rocket immunoelectrophoresis a[illegible] titation Special tubes containing thrombin and epsilonaminocaproic and [illegible] were used for blood samples intended for FDP quantitation.

Fibrinolytic activity was estimated by the fibrin plate method (Astrup [illegible] 1952) 30 μl fresh aqueous humour or serum was applied to the [illegible] plate After 18 h of incubation at 37°C the plates were inspected for areas of [illegible]

α macroglobulin $α_1$ antitrypsin plasminogen C_3 complement were quan[illegible] rocket immunoelectrophoresis in which the protein was precipitated in an a[illegible] taining the specific antibody The size of the rocket is directly proport[illegible] concentration of the antigen in question (Laurell 1966) Antibodies were [illegible] from Behringswerke W Germany The results were expressed as per cent of [illegible] concentration found in pooled normal serum from healthy donors 10 men [illegible] women

Serum samples were applicated in a 1 20 dilution while the aqueous humour [illegible] tested undiluted to bring the protein concentration within the discrimina[illegible] of the method In our hands the method had a coefficient of variation of 9%

Fibrinogen degradation products (FDP) were measured by the Thrombo-W[illegible] test using latex particles coated with antihuman fibrinogen (Wellcome [illegible] Beckenham England) The test is semiquantitative The serum was diluted [illegible] the test agglutination occurs if the concentration of FDP exceeds [illegible] mg/l. The [illegible] was tested undiluted

Results

The content of the various factors in the aqueous is shown in Table I [illegible] be seen that the concentration of α macroglobulin is almost the same [illegible] aqueous from patients with Fuchs dystrophy uveitis and in the secondary aqueous from the cataract patients whereas it is only about half of this the primary aqueous of the cataract group The difference between the aqu[illegible] from the Fuchs patients and the primary aqueous from the cataract pa[illegible] is however not significant (t = 46) $α_1$ antitrypsin is found to be increased [illegible] patients with Fuchs dystrophy in uveitis and in the secondary aqueous from cataract patients The difference between Fuchs dystrophy and the primary aqueous from cataract patients is in this case significant (t = 2.49 $P <$ [illegible] The concentration in the secondary aqueous from the cataract pa[illegible] between that of the primary aqueous and the aqueous concentration from [illegible] Fuchs dystrophy patients The concentration in the patients with a[illegible] uveitis is approximately 9 times more than the primary aqueous of the cata[illegible] patients

Table I

relative concentrations in aqueous humour of factors of the fibrinolytic system -omplement factor C_3 as determined by immunoelectrophoresis using pooled nor mal serum as reference.

	Fuchs dystrophy	Cataract Primary aqueaus humour	Cataract Secondary aqueous humour	Anterior uveitis
acroglobulin	0 14	0 08	0 1[illegible]	0 15
SEM	0 04 (n = 9)	0 01 (n = 17)	0 02 (n = 6)	0 0[illegible] (n = 2)
ntitrypsin	1 [illegible]0	0.30	0 67	[illegible] 60
SEM	0 36 (n = 9)	0 04 (n = 17)	0 18 (n = 6)	2 40 (n = 2)
minogen	0 41	0 07	0 28	2 8[illegible]
SEM	0 [illegible] (n = 9)	0 01 (n = 1[illegible])	0 16 (n = [illegible])	0 4[illegible] (n = 2)
omplement	0 19	0 09	0 2[illegible]	1 70
SEM	0 06 (n = 8)	0 02 (n = 16)	0 0[illegible] (n = [illegible])	0 30 (n = [illegible])
	negative (n = 8)	negative (n = 16)	negative (n = [illegible])	negative (n = 2)
n plates	negative (n = 8)	negative (n = 16)	negative (n = [illegible])	negative (n = [illegible])

Plasminogen is increased in the Fuchs dystrophy patients in anterior uveitis l in the secondary aqueous of the cataract patients The concentration in secondary aqueous also lies between that of the primary aqueous of aract patients and the aqueous from patients with Fuchs dystrophy The ference between Fuchs and the primary aqueous from cataract patients is t significant (t = 1 54)

C_3 complement is also increased in the patients with Fuchs dystrophy in terior uveitis and in the secondary aqueous of cataract patients The dif ence between the primary aqueous from cataract patients and the aqueous om Fuchs patients is not significant (t = 1 58)

Neither FDP nor fibrinolytic activity could be demonstrated in any of the oups

The serum content of the various factors is shown in Table II It is to be ted that all the patients underwent venepuncture only once The serum

values for the six patients in whom secondary aqueous was [illegible] therefore also included in the values for the primary aqueous group

In the case of α macroglobulin and α_1 antitrypsin it can be seen [illegible] values are almost identical for the cataract group and the [illegible] whereas a non significant tendency towards higher values is [illegible] patients with Fuchs dystrophy

In the case of pla minogen and C_3 complement higher [illegible] found in patients with Fuchs dystrophy and anterior uveitis than [illegible] with cataract (not significant)

FDP could be demonstrated in the serum (> 10 mg/l) in six out of [illegible] patients with Fuchs dystrophy In the cataract group FDP was [illegible] in three out of 16 patients This difference is significant (Fischer $P <$ [illegible] Fibrinolytic activity could not be demonstrated in the serum in [illegible] patient groups

Table II

The serum concentration of various factors of the fibrinolytic system [illegible] percentage of the normal serum concentration

	Fuchs dystrophy	Cataract Primary aqueaus humour	Cataract Secondary aqueous humour	A [illegible]
α macroglobulin $\bar{x} \pm$ SEM	114 1 8 9 (n = 8)	96 2 10 3 (n = 16)	93.3 10 9 (n = 6)	93 0 10 [illegible]
α_1 antitrypsin $\bar{x} \pm$ SEM	96 2 5 3 (n = 8)	91 1 3 4 (n = 16)	9[illegible] 6 5 5 (n 6)	[illegible] 3 3 [illegible]
Plasminogen $\bar{x} \pm$ SEM	71 [illegible] 6 5 (n = 8)	66 0 3 1 (n = 16)	67 8 6 4 (n 6)	[illegible]
C_3 complement $\bar{x} \pm$ SEM	128 2 6 5 (n = 8)	115 5 3 5 (n = 16)	109 4 6 6 (n 6)	[illegible]
FDP (≥ 10 mg/l)	positive 6 negative 2	positive 3 negative 13	negative 6	neg[illegible]
Fibrin plates	negative 8	negative 16	negative 6	neg[illegible]

Discussion

- e are no studies in the literature concerning the concentration of the ›rs mentioned in the aqueous humour of humans It is therefore somewhat tionable to consider the aqueous of cataract patients as being normal For ·al reasons this is the most normal material that can be obtained In the ent study a comparison is made between cataract patients and patients with is dystrophy and as described cataracts were also present in the Fuchs ents It must therefore be reasonable to relate the difference in the com tion of the aqueous to the difference between cataract patients and patients ı Fuchs dystrophy The rocket immunoelectrophoresis is sensitive to ein concentrations down to 0 3 mg/l Serum samples were diluted 1 20 in er before application while the aqueous was tested undiluted This means concentrations down to 0 05 % of serum values could be examined with ainly The difference in the aqueous between these two groups of patients nificant for α_1 antitrypsin) could be due to the effect of the pathological othelium of the Fuchs patients However it is also possible that the aqueous Fuchs patients has a toxic effect on the cornea endothelium The last ement appears to be confirmed by the composition of the aqueous in the ients with uveitis In uveitis there is inflammation of iris and ciliary body h a breakdown of the blood aqueous barrier and cornea oedema is seen develop in these patients The alteration in the examined factors in the ieous is greater in the patients with anterior uveitis than in the patients h Fuchs dystrophy In anterior uveitis the oedema develops acutely as posed to the oedema in Fuchs dystrophy which develops over the course of eral years The patients with anterior uveitis had an increased level of complement in the serum (not significant) whereas the other factors in the um did not differ from the patients with cataract FDP was negative in se patients This suggests a local reaction in the anterior segment of the eye the Fuchs patients there was a tendency towards a higher serum con ntration of the factors examined and there was an significantly increased DP concentration This could be explained if Fuchs endothelial dystrophy curs as a part of a generalised disease with alterations in the fibrinolytic stem In this way the reducing effect of tranexamic acid on corneal oedema Fuchs dystrophy is understandable The present study suggests that altera ons in the composition of the aqueous for local or more general reasons can use incompensation of the cornea endothelium This situation must for cample be considered when placing clip lenses in younger patients

Material and Methods

Sixteen cataractous lenses extracted from eyes with clinical FEC syndrome ... cataractous lenses from otherwise normal eyes were subjected to scanning ... microscopy and confirmatory transmission electronmicroscopy after having been ... to a variable degree of either α chymotrypsin or collagenase or a combination ... Some lenses were untreated and some were sham treated serving as ... controls (Table I)

Following extraction the lenses were immediately put into a small beaker ... sterile perfusion medium without enzymes The lenses were transferred ... to a platinum grid and perfused with enzyme solution at a rate of one d... by means of a peristaltic pump The whole experiment was carried out in ... at 37°C and lasted from 45 to 120 min Following the perfusion, the lenses ... for 24 h at 5°C in 2 5 % glutaraldehyde buffered to pH 7 4 with Hepes buffer ... slice was then cut and processed according to earlier description (Seland 19... and the two remaining parts were bisected producing four quarters of ... were processed for SEM according to an earlier described method (Seland ... TEM sections were cut in an LKB microtome and examined in Philips EM 30... while the tissues for SEM were processed through a critical point apparatus ... coated with approx 17 nm gold in a sputter apparatus (Polaron) (Roth & ... and examined in a Philips PSEM 500 microscope

The following enzyme concentration were used α chymotrypsin (Novo/Ame... units per 100 ml perfusion fluid and collagenase (Sigma type III and ... per 100 ml perfusion fluid The perfusion fluid consisted of Ringer acetat ... pH 7 4 with Hepes buffer and 4 mg gentamycin (Garamycin®) added per ...

Control lenses

Two lenses with clinical fibrillopathia (Nos 1 and 2) and two lenses with ... senile cataract (Nos 3 and 4) were processed for microscopy without having ... subjected to any perfusion experiments In addition two lenses extracted from ... eyes (Nos 5 and 6) and two cataractous lenses (Nos 7 and 8) were ... for 60 to 90 min in an experimental procedure identical to the rest of ... but without addition of any kind of enzymes These eight lenses were considered ... control group

α chymotrypsin experiments

Two FEC lenses (Nos 9 and 10) and two normal cataractous lenses (Nos 11 ... were subjected to enzyme action for 60 to 90 min

Collagenase experiments

Three FEC lenses (Nos 13–15) and three normal cataractous lenses (Nos 16–1... subjected to collagenase actions for periods varying from 60 to 90 min.

Combined enzymatic action

Seven FEC lenses (Nos 19–25) and three normal lenses (Nos 26–... were ... to a perfusion with a combination of α chymotrypsin and collagenase for 45 to 1...



Fig 1

Anterior surface of untreated FEC control lens
The peripheral granular band (gb) is seen as an irregular layer in the periphery
Anterior pole towards upper right corner Bar 100 μm
and C. Busacca bushes from granular band of two different untreated FEC lenses
B there is a flattened appearance of the fibrillar network with small melanin
pherules (arrows) and smooth red blood cells In C the Busacca bushes have a highly
fibrillar appearance Bars 10 μm

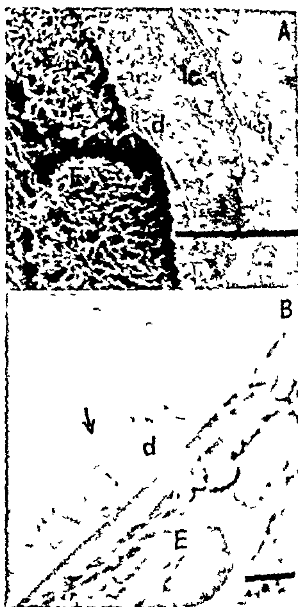


Fig 9

A Sham treated lens
Section through lens capsule in the area of zona germinative Highly porous ... of epithelial cells (F) with overlying *lens capsule (lc)* containing fibrillar deep ... with tentacles and incusion bodies *Bar 10 µm*
B Phasecontrast of semithin section from zona germinative after *collagenase* treatment Only remnants of capsula propria (arrow) are seen on an intact discoid plane of ... layer (d) E– epithelial cell Bar 9 µm

Results

ol experiments

najor differences could be found between the surface ultrastructure of the untreated and sham treated lenses They had a similar distribution of fibrollopathia material on the anterior lens surface with a central disc a peripheral granular band (Fig 1) The central discs however were not lly conspicuous in all lenses The FEC material was usually rife on the lar threads in the equatorial region In the sham group the anterior ule had occasionally linear breaks in the equatorial region which might se discoid plaques of deep layer with pits and tentacles (Fig 2 A) (Seland) Large magnifications of the surface material from the peripheral band central discs in FEC lenses showed a strikingly similar intertwined fibrillar cture (Fig 12 A B C D) The material in the peripheral granular band ered only from the central disc by increased presence of spherical melanin nules (Fig 1 B) The thickness of the fibrillar components were measured 50–80 nm in the SEM preparations The lengths were difficult to judge as fibrils were twisted in a complex meshworks but at least 3 μm could be erned

The non FEC lenses did not show any of the above mentioned surface tures The zonular threads were as a rule well preserved both in untreated l sham treated lenses

hymotrypsin experiments

ither the FEC material from central disc nor the peripheral band were pearently affected by α chymotrypsin (Fig 3 A) The zonular threads covered th FEC material were also more resistant to the enzyme action than normal nules (Fig 3 B)

In the normal lenses 90 min of perfusion left a completely smooth and bald rface with no traces of zonular apparatus but the different textures of the terior and posterior capsules were separated by a distinct equatorial line

ollagenase experiments

he collagenase treated FEC lenses had an intact peripheral band but no entral disc on the anterior capsules (Fig 4 A) The zonular apparatus could e found unaffected while the capsula propria centrally and between the olunar threads was heavily eroded (Fig 4 B) In lens no 13 a linear rupture n the zonular lamina had occurred in the preequatorial region and the enzyme ad gained acces to the underlying capsule propria digesting the superficial art and revealing large circular area with diameters varying from 100–150 μm

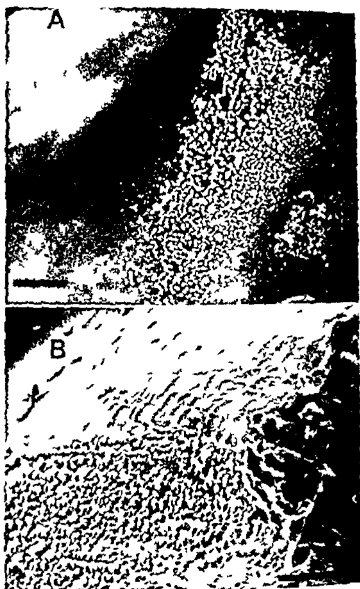


Fig 3

α chymotrypsin experiment

A The peripheral granular band is not removed by the enzyme

B FEC *material* seem to protect some zonular threads from the action of the en

Anterior poles towards left Bars 100 μm

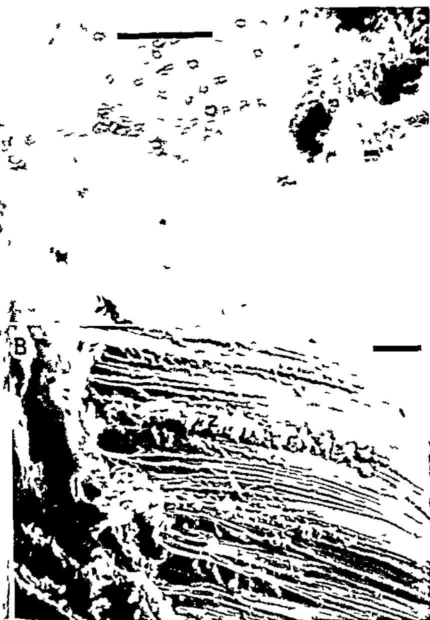


Fig 4
Collagenase experiment
\ The peripheral granular band (gb) is unaffected by enzyme Bar 100 μm Inset Enlargement of the Busacca bushes shows a normal fibrillar structure Bar 1 μm
B Zonular threads (z) are not destroyed by collagenase Bar 100 μm

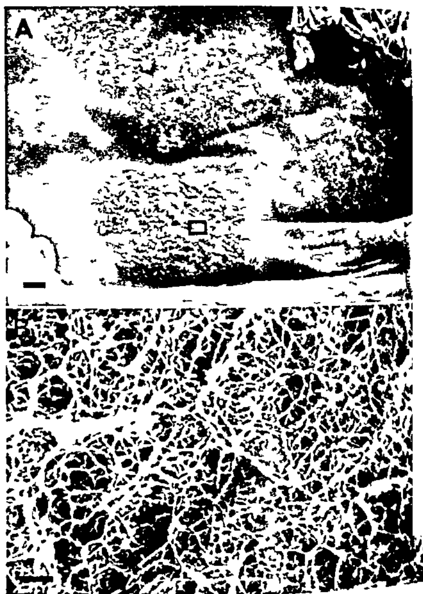


Fig 5

Collagenase experiment

A Preequatorial region where zonular lamina has ruptured Circular area of [illegible] layer containing intertwined fibrils Bar 10 μm

B Enlargement of rectangular area in A The fibrils have a thickness up to [illegible] length up to 3 μm Bar 1 μm

esponding in site and size to large discoid plaques of deep layer (Fig 5 A) h magnifications of their surface revealed an intricate configuration isting of interwoven fibrils with diameters from 50–80 nm (Fig 5 B and)

hin and ultrathin sections through these circular areas in FEC lenses irmed that they represented discoid plaques of deep layer with intact ils and a variable degree of residual capsule (Fig 2 B)

s in the α chymotrypsin treated lenses the normal cataractous lenses in the agenase experiments had a clearly defineable equator parallel demarcation



Fig 6

Combined enzymatic experiment on FEC lens

obble stone sector (cr) in the anterior lens capsule ending in a distinct laminar edge (e) Bar 100 μm

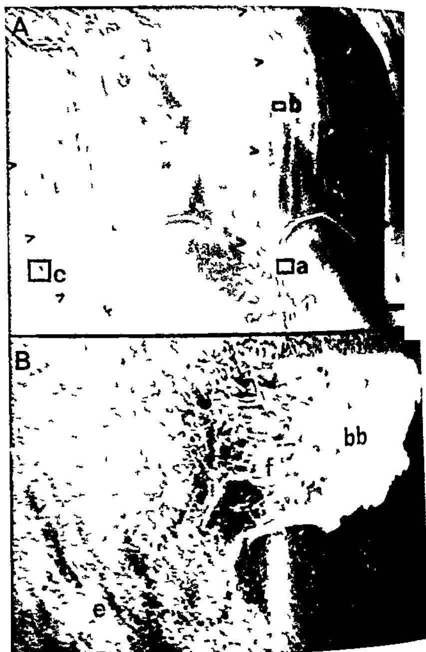


Fig 7

Combined enzymatic experiment on FEC lens

A Anterior lens capsule with three cobble stone sectors (arrows) [illegible]
projections a b c Enlarged areas Bar 100 µm.

B (box a in fig A) Busacca bush (bb) at the edge of the lamina [illegible]
structure (f) of the bush and its connection to the cobble stone elevation (e) [illegible]

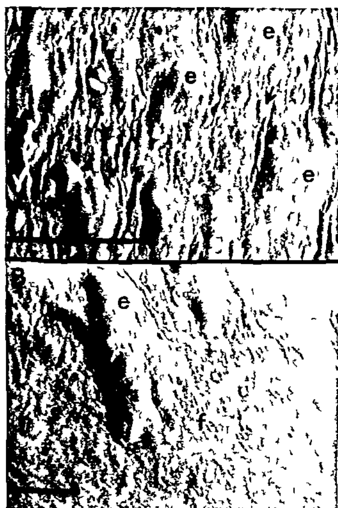


Fig 8
Combined enzymatic experiment on FEC lens
Enlarged section from fig 7 A
(box b) Midsection of cobble stone sector Note the cobble stone (e) parallel linear structures (l) and the globular formations (arrows) Bar 10 μm
(box c) Large elevations (e) on the edge of the lamina surrounded by finely fibrillar material (f) Bar 10 μm

ne separating the anterior lens capsule from the posterior Remnants of the nular threads were also observed The hexagonal outline of the lenticular lls could easily be depicted through the remaining thin film of capsular aterial in some lenses

Ehlers 1969) The α chymotrypsin easily degrades the normal zon[illegible] Zonular threads heavily infested with FEC material however were [illegible] affected by the enzyme Whether the FEC material constituted a [illegible] barrier or the zonular apparatus had a pathological structure [illegible] determine Davanger & Pedersen (1975) have demonstrated that [illegible] Busacca bushes constitute a considerable barrier for large molecules and [illegible] therefore not inconceivable that they may afford a true protection [illegible] enzymes

The experiments also showed that the central discs were readily [illegible] collagenase acting on the underlying capsule In addition to the [illegible] that no characteristic changes have been described in the central [illegible] capsula propria this is strong evidence for the view that the [illegible] probably is only a secondary phenomenon caused by the material [illegible] to this location by the aqueous humour The peripheral granular band [illegible] however unaffected by any single enzyme signifying a strong [illegible] anchorage

The peripheral granular band and the zonular excrescences were [illegible] removed by a relatively short concerted action of both enzymes [illegible] the peripheral edge of this band which appeared as a serrated lamina

In this relatively superficial layer of the lens capsule several distinct [illegible] of enzyme resistant capsular inclusions were demonstrated

The most conspicuous were smooth rounded cobble stone elevations [illegible] diameters from 2 to 12 μm which were arranged in radial sectors [illegible] teeth like projections of the laminar edge These elevations probably [illegible] some of the large inclusions in the FEC lens capsule earlier described as [illegible] and dots (Bertelsen et al 1964)

The two other formed bodies observed in this layer were also present [illegible] more conspicuous way in the epithelium near stratum where relatively [illegible] worm like spindleshaped structures with diameter of about 800 nm and [illegible] exceeding 3 μm were found The only difference between the appearance [illegible] the superficial cobble stone stratum and the deep epithelium near [illegible] were a high degree of directional uniformity in the former where the [illegible] crescences were parallel and radially arranged (Fig 8 B) The structures [illegible] in the capsule were curved in a haphazard fashion (Fig 9 A) These [illegible] probably represent the normal capsular inclusion bodies found with [illegible] age in all capsules (Dark et al 1969 Seland 1974) The superficial [illegible] shaped structures may possibly afford the necessary deep anchorage [illegible] cipitation cores for the fine fibrils of the Busacca bushes Their uniform [illegible] orientation [illegible] be explained as a result of the sentripetal growth [illegible] of the capsu[illegible] On the other hand they might represent [illegible] artifact

ird conspicuous component of the FEC capsules were small globular res seen both in the superficial layer and in the epithelium near stratum probably represent the annular bodies demons rated in the capsula a (Bertelsen et al 1964)

ly fibrillar material (FEC material) could be identified on the lens (*Fig 12 A B C D) and in the superficial* cobble stone stratum (Fig s well as a component of the deep layer of discoid plaques (Fig 9 A E and G) The fibrils at all sites had dimensions of the same order of tude i e about 50–80 nm thick and length varying up to 5 μm The st concentrated area found to be covered by the fibrillar surface of a d plaque was about 10 μm corresponding to a cell diameter (Fig 9 A) g into consideration a coat thickness of 15–20 nm they correspond to the opathy material observed earlier (*Bertelsen et al 1964* and Sugar et aL

some lenses wells and holes could be found in the preequatorial with small rounded structures inside (Fig 11 A) These probably repre he Hassall Henle like warts sometimes found in this region (Seland

ed on the present findings the phenomenon of FEC may be explained in llowing way

e to some unknown stimulus cells in the zona germinativa of the lens orming pathological fibrillar material In addition the cells are stimulated oduce increasing amounts of the coarser formed bodies of the normal lens capsule *As the major part of the capsular production occurs in the germinativa* and as a continuous *resorbtion probably occurs in the central* not covered by lamina zonularis the growth turnover results in a sliding port of capsule from the production site towards the resorbtion area ing with it all kinds of inclusions and pathological capsular substances n[d] 1976) As long as production of pathological capsular components is the growth transport will remove all pathological components from the elium and in due course release it on the surface of the lens If the logical fibrillar production is accelerated large aggregation will form on roduction site in the shape of discoid plaques of deep layer

production of pathological capsules seem to occur in 20–30 preequatorial rs the concentrating effect of the centripetal transport may cause the logical capsular substance to form preequatorial stria (Bartholomew 1971) a sheath or lam*ina when it appears in the smaller resorbtion area*

e resorbtion process of the aqueous humour aided by the mechanical action e pupillary border releases the capsular components Some fibrils how have firm anchorage in capsular meshwork or to the coarser spindle

Ehlers 1969) The α chymotrypsin easily degrades the normal zonula [illegible] Zonular threads heavily infested with FEC material however were [illegible] affected by the enzyme Whether the FEC material constituted a pr[illegible] barrier or the zonular apparatus had a pathological structure, is not [illegible] determine Davanger & Pedersen (1975) have demonstrated that the [illegible] Busacca bushes constitute a considerable barrier for large molec[illegible] it is therefore not inconceivable that they may afford a true protection against enzymes

The experiments also showed that the central discs were readily removed [illegible] collagenase acting on the underlying capsule In addition to the observation that no characteristic changes have been described in the central [illegible] capsula propria this is strong evidence for the view that the cent[illegible] probably is only a secondary phenomenon caused by the material bein[illegible] to this location by the aqueous humour The peripheral granular band was however unaffected by any single enzyme signifying a stro[illegible] anchorage

The peripheral granular band and the zonular excrescences were, [illegible] removed by a relatively short concerted action of both enzymes lea[illegible] the peripheral edge of this band which appeared as a serrated lam[illegible]

In this relatively superficial layer of the lens capsule several dist[illegible] of enzyme resistant capsular inclusions were demonstrated

The most conspicuous were smooth rounded cobble stone eleva[illegible] diameters from 2 to 12 μm which were arranged in radial sectors [illegible] teeth like projections of the laminar edge These elevations probably [illegible] some of the large inclusions in the FEC lens capsule earlier described as [illegible] and dots (Bertelsen et al 1964)

The two other formed bodies observed in this layer were also prese[illegible] more conspicuous way in the epithelium near stratum where rela[illegible] worm like spindleshaped structures with diameter of about 800 nm and exceeding 3 μm were found The only difference between the appe[illegible] the superficial cobble stone" stratum and the deep epithelium ne[illegible] were a high degree of directional uniformity in the former where [illegible] crescences were parallel and radially arranged (Fig 8 B) The struct[illegible] in the capsule were curved in a haphazard fashion (Fig 9 A) These [illegible] probably represent the normal capsular inclusion bodies found wi[illegible] age in all capsules (Dark et al 1969 Seland 1974) The superficial [illegible] shaped structures may possibly afford the necessary deep anchorage [illegible] cipitation cores for the fine fibrils of the Busacca bushes Their unif[illegible] orientation may be explained as a result of the sentripetal growth [illegible] of the capsule (vide infra) On the other hand they might repr[illegible] artifact

ven E (1935) *Om den senile exfoliasjon av linsekapselen* (Vogt) Særlig dens for old til glaucoma simplex Diss Grondahl & Sons bogtrykkeri Oslo
ven I (1966) Exfoliation syndrome A histological and histochemical study *Acta phthal (Kbh) 44* 790–800
use U & Tarkkanen A (1978) Cataract and Pseudoexfoliation A Clinicopathological Study *Acta ophthal (Kbh) 56* 329–334
adberg J G (1917) Kliniska undersokningar over depigmenteringen av pupillar anden och genomlysbarheten av iris vid fall av ålderstarr samt i normale ogon hos amla personer *Diss Helsingfors*
lling B (1973) Untersuchung uber das Verhaltnis zwischen Iridocyclitis und laukom *Acta ophthal (Kbh) 1* 97–130
hsteiner K (1978) Zur Klinik des Linsekapselhautchenglaukoms *Zeitschr f Augenheilk 66* 103–104
gvold A (1969) Electronmicroscopy of the wall of the iris vessels in eyes with and without exfoliation syndrome (pseudoexfoliation of the lens capsule) *Virchows Arch Path Anat 348* 328–341
J & Flood P R (1978) A simple method for the determination of thickness and rain size of deposited films as used on non conductive specimens for scanning electron microscopy *Journal of Microscopy 112* 359–364
land J H (1974) Ultrastructural changes in the normal human lens capsules from birth to old age *Acta ophthal (Kbh) 52* 688–706
land J H (1976) Production of human lens capsule illustrated by a case of chronic lenticular chalcosis *Acta ophthal (Kbh) 54* 301–310
land J H (1978) The ultrastructure of the deep layer of the lens capsule in fibrillopathia epitheliocapsularis (FEC) so called senile exfoliation or pseudoexfoliation A Scanning Electron Microscopic Study *Acta ophthal (Kbh) 6* 335–348
gar H S (1976) The Exfoliation Syndrome Source of Fibrillar Material on the Capsule *Surv Ophthal 21* 59–64
gar H S Harding C & Barsky D (1976) The Exfoliation Syndrome *Annales of ophthalmology 8* 1165–1181
nde O A (1956) On the so called senile exfoliation of the anterior lens capsule *Acta ophthal (Kbh) suppl 45*
akei Y & Smelzer G K (1975) Electron Microscopic Studies on Zonular Fibers II Changes of the Zonular Fibers after Treatment with Collagenase α Chymotrypsin and Hyaluronidase *Albrecht v Graefes Arch Ophthal 191* 1 3–173
arkkanen A (1962) Pseudoexfoliation of the lens capsule A clinical study of 418 patients with special reference to glaucoma cataract and changes of the vitreous *Acta ophthal (Kbh) Suppl 71*
ogt A (1925) Eine neues Spaltlampenbild des Pupillengebietes Hellblauer Pupillensaumfilz mit Hautchenbildung auf der Linsenvorderkapsel *Klin Mbl Augenheilk 75 (II)* 1–12
ogt A (19 6) Eine neues Spaltlampenbild Abschilferung der Linsenvorderkapsel als wahrscheinliche Ursache von senilen chronischen Glaukom *Schweiz med Wschr 6* 413–415

Author's address
Johan H Seland,
Department of Ophthalmology
Olf Haukeland Sykehus
Norway

The Department of Ophthalmology Hjørring Sygehus Denmark
(Heads M Lowes and N Vedel Jensen)

Short communication

VITREOUS CUTTER IN ANTERIOR SEGMENT SURGERY

BY

MARTIN LOWES and NIELS VEDEL-JENSEN

Experience has been gained in operating upon a variety of a[illegible] segment disorders using a Grieshaber vitreous cutter The results and complications are briefly discussed

Key words vitreous cutter – lensectomy – anterior segment reco[illegible]

In 1971 Machemer introduced the vitreous infusion suction cutter [illegible] whereby ocular tissue could be cut removed and replaced through a [illegible] tube inserted via the pars plana The technique was primarily [illegible] use in the posterior segment of the eye (Machemer et al 1971) but has also been applied in a wide variety of pathological conditions in the [illegible] segment of the eye with favourable results (Kanski & Crick 19[illegible] [illegible] Machemer 1978 Lullwitz & Sautter 1978) Our experience with this tech[illegible] in 19 patients with various anterior segment disorders is presented.

Material and Methods

A total of 19 patients underwent surgery and of these there were seven cas[illegible] cataract including three cases of congenital cataract two cases of tra[illegible] one case of subluxated congenital cataract and one case of secondary cataract following chronic anterior uveitis Nine cases underwent anterior segment [illegible] either for removal of pupillary membranes removal of capsulo lenticular [illegible] for pupillotomy There was one case of vitreous opacities following [illegible] extraction and two cases of aphakic bullous keratopathy

Received December 11 197[illegible]

Operati e technique The operation was performed under the operating microscope ng a Greishaber vitreous cutter the cutting head of which consists of a rotatory illating four bladed knife contained within a metallic tube. The material to be is aspirated through a small hole directly adjacent to the knife blades Inflow of id into the anterior chamber occurs through the central tube and is controlled by ivity The anterior chamber was opened at the limbus and the vitreous cutter erted while keeping the incision as watertight as possible The cutting was control by the surgeon and aspiration performed by the assistant In the two cases of umatic cataract and in eight of the nine cases of anterior segment reconstruction operation was performed as a new technique designed to remove the scaffolding ich had previously caused repeated opacification following operations with a dis sion knife or Vannas scissors

Results

Lensectomy The technique proved to be satisfactory in removing lenticular sue especially when this was soft In the case of the secondary cataract e lens nucleus was too hard to be aspirated and the incision was therefore tended and the nucleus removed by extracapsular extraction Improvement visual acuity occurred in all seven cases The preoperative visual acuity as 0 9 or less in all cases The postoperative visual acuity was 0 3 in three ses and better than 0 5 in the remaing four cases Transient glaucoma and permanent sector striate keratitis occurred in one case of traumatic cataract ost probably due to prolonged operative intervention The eye has since tained a visual acuity of 1 0

Anterior segment reconstruction This was undertaken in nine eyes including ven cases with capsulo lenticular remnants and two cases with pupillary embranes where pupil enlargement was performed In two cases the pupil ry membranes were of such a thickness that they could not be directly pirated into the cutting port and had to be cut into smaller pieces using annas scissors The preoperative visual acuity was counting fingers or less six cases 0 1 in two cases and 0 4 in one case Postoperatively considerable nprovement had occurred in five cases with a visual acuity of 0 3 in three ises 0 5 in one case and 0 7 in one case In the remaining four cases visual cuity was more or less unchanged and retinal detachment occurred in two f these cases Transient striate keratitis occurred in four eyes In one eye a uck fibrous membrane could not be removed

Bullous keratopathy In the two cases of bullous keratopathy operation ailed to remove the cornea oedema. One case developed a transient glaucoma

Vitreous opacities In the sole case of aphakic vitreous opacities anterior itrectomy resulted in a visual acuity of 1 0

Discussion

The use of the vitreous cutter provides an interesting new devel... surgery of the anterior segment of the eye Tissue is removed ... under microscopic control and the anterior chamber is maintained th... the procedure In the present series the technique was successfully ... out of 16 cases of cataract and anterior segment reconstruction. In ... cases where the lens was hard or the membranes were thick ... difficulty was experienced with the rotatory cutter This led to p... operations with excessive fluid inflow This undoubtedly caused ... cell damage with subsequent transient striate keratitis in four cases ... manent sector striate keratitis in one case The two cases of trans... seemed to occur on the basis of a type of malignant glaucoma and ... due to excessive fluid forming pockets in the vitreous Retinal d... occurred in two cases In this respect retinal detachment was also re... Litwin (1978) following the use of the Kaufman vitrector I where ... action led to spooling of the vitreous The instrument has since been ... and the Kaufman II has a guillotine action In our opinion the ... constitutes a significant advance in anterior segment surgery How... rotatory action is not effective enough for anterior segment surgery ... complications encountered are to a large extent felt to be due ... efficiency of this rotatory action Accordingly a further series is plan... a vitreous cutter with a guillotine action

References

Kanski J J & Crick M D P (1977) Vitrectomy technique in anterior segm... *Brit J Ophthal 61* 209–215

Litwin R L (1978) Correspondence Retinal dialysis secondary to the use o... man vitrector *Amer J Ophthal 85* 8/9–880

Lullwitz W & Sautter H (1978) Über erfahrungen mit dem vitrektor bei d... lung des nachstars *Klin Mbl Augenheilk 1/2* 54–58

Machemer R Buettner H & Norton E W D (1971) Vitrectomy A... approach *Trans amer Acad Ophthal Otolaryng 75* 813–820

Treister G & Machemer R (1978) Pars plana approach for pupillary ... *Arch Ophthal (Chicago) 96* 1014–1016

Author's address

Martin Lowes
Ulvegravene 10
9800 Hjørring
Denmark

Department of Experimental Ophthalmology (Head C E T Krakau)
University Eye Clinic Lund Sweden and
the Dalby Health Service Research Center (Head A Norden)
Vardcentralen Dalby Sweden

Short communication

FLICKER COMPARISON OF FUNDUS PHOTOGRAPHS

A technical note

BY

BO BENGTSSON and C E T KRAKAU

A method for comparison of two fundus transparencies by means of alter nating flickering light is described Spurious differences between the pictures are reduced by making the exposures in a fixed position of the pulse cycle Small haemorrhages are readily detected Pulse variations of the vessels and changes in size of cup and disc can be measured

Key words papillary measurements – pulsations

peated photography of the eye fundus is a method to be recommended when development of pathological processes has to be followed but subtle changes difficult to estimate by simple examination of the pictures The method lled Stereochronoscopy developed by Goldmann & Lotmar (1977) deals th the problem of comparing fundus photographs taken on different occa ons

The method to be described here is intended not only to make possible the servation of minute changes between pictures but also to measure them he principle adopted is well known from other fields such as astronomy and entgenology (Edholm 1976)

eceived February 8 19 9

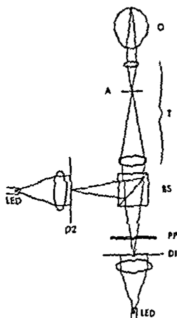


Fig 1

Flicker comparator O observer's eye T magnifying tube BS beam [illegible] parallel glass plate D1 D2 transparancies LED light emitting diodes A [illegible]

Method

The two transparencies to be compared are fitted into an instrument [illegible] in Fig 1 They are illuminated from behind and by use of a beam [illegible] they are seen superimposed and magnified 10–20 diameters in the [illegible] One of the pictures is placed on a cross table the other mounted in a [illegible] setting and it is therefore possible to adjust identical pictures so [illegible] are seen in complete coincidence through the tube In order to [illegible] discrepancies between pictures taken on different occasions of the [illegible] ground the light sources which are light emitting diodes LEDs [illegible] nately at frequencies which can be set from 1/2 Hz to about 20 Hz [illegible] which have changed position somewhat between the two occasions [illegible] seen fluttering whereas the rest of the picture remains stable

In order to make small deviations measurable a plane parallel [illegible] glass plate (PP) is mounted between one of the pictures and the beam [illegible] This can be rotated around a horizontal axis thus producing a [illegible] one image The relation between the rotation of the mirror and the [illegible] placement of the image is approximately linear for small angles

The light intensity of the LEDs can be balanced so as to compensate for dif -nces in exposure

urces of spurious variation

rious sources of error which may cause spurious differences between the tures are discussed by Goldmann & Lotmar (19/8) In order to avoid paral three dimensional structures for example excavated discs have to be photo aphed from the same direction and through the same part of the optic media .h time By using the procedure for photography through a natural pupil engtsson & Krakau 1979) these conditions are largely fulfilled Centering the pupil in relation to the camera and a correct distance to the eye are the ns of this technique

The direction of the gaze is checked by the location of the disc on the pictures

lsations

or the study of the pulsatile variations a device was constructed by means of hich an exposure can be made at a predetermined moment of the pulse cycle a small ECG amplifier is connected to the patient and the R peak is allowed to start a time circuit which in its turn determines the moment when it is pos ble to release the flash Thus when the exposure lever is pushed the camera iutter is opened but the flash is not released until a pre set delay (0 1 to 1 sec fter an R peak) has elapsed.

Discussion

ets of pictures were taken on a normal person with clear mediae Keeping the djustment as constant as posible and varying the moment of exposure we ould see the configuration variation of the vessels at the papilla when com aring pictures in different pulse phases Displacements can be determined on harp pictures with an s d of $< 2\mu$ a degree of precision which makes the tudy of pulsation effects possible The veins compressed during systole dilated o maximal diameter about 0 4 secs after the R peak The amplitude was of he order 40μ The artery showed caliber changes too but to a smaller degree – about 5μ There was also serpentine movements – The measurements were made at a flicker frequency of 1–5 Hz

On *Glaucoma cases* haemorrhages occurring on the papilla were detected without difficulty A haemorrhage present on one picture but not on the other is very striking coming and going with flickering of the lights

Also the extension of the excavations can be detected at an [illegible] Small distinctly demarcated vessels on the papillary substance are [illegible] landmarks

Some small errors of adjustment (or by change of position between [illegible] ment and exposure) causing differences between pictures can seldom be [illegible] pletely avoided Since parallactic errors displace corresponding [illegible] same direction which is not the general rule in the case of true [illegible] shape it may be possible to avoid spurious differences by careful [illegible] The use of more than one exposure on each occasion so that [illegible] correspondence can be chosen is also of value at evaluation. Furth[illegible] of exposures locked in a fixed pulse phase is recommended in order to [illegible] very small changes A material of repeated exposures in the fixed [illegible] on increasing glaucomatous excavations is now being collected with [illegible] elucidating the efficiency of the method in practice

References

Bengtsson B & Krakau C E T (1979) A simple routine for optic disc [illegible] through a natural pupil *Acta ophthal (Kbh)* 57 151–154

Bengtsson B & Krakau C E T (1977) some essential optical features of the [illegible] fundus camera *Acta ophthal (Kbh)* 55 123–131

Edholm P (1972) Apparat for jamforelse av två rontgenbilder Svensk [illegible] medicinsk radiologi Forhandlingar nr 1 p 10

Goldmann H & Lotmar W (1977) Rapid detection of changes in the [illegible] Stereo chronoscopy *Albrecht v Graefes Arch klin exp Ophthal* [illegible]

Goldmann H & Lotmar W (1978) Rapid detection of changes in the optic [illegible] chronoscopy II Evaluation technique influence of some physiologic [illegible] follow up of a case of choked disc *Albrecht v Graefes Arch klin exp O*[illegible] [illegible]63–2[illegible]7

Authors addresses

C E T Krakau Professor M D
Department of Experimental Ophthalmology
University Eye Clinic
S 221 85 Lund
Sweden

Bo Bengtsson M D
Vårdcentralen
S 240 10 Dalby
Sweden

Harold A & Slatt Bernard J The Ophthalmic Assistant Fundamentals and clinical practice 3ed pp 594 853 ill Mosby St Louis 1976 Prize $ 24 95

. new edition has been expanded with two chapters concerning soft contact lenses ctic questions after each chapter actual new topics such as night vision goggles in itis pigmentosa prosthetic aids for the blind (TV cameras under motor control con) intraocular lenses phacoemulsification automatic refractors AO non contact meter computer systems for hard contact lens fitting hard gas permeable contact 's pilocarpine spray bottle and ocusert

he text is easy to understand vivid and interesting The artistic figures and photo ihs are very instructive The book covers all aspects of interest for the ophthalmic itant inclusive clinical practice tonography eye photography optic illusions rmacology refraction contact lenses eye disorders classification of urgency aseptic nique and glossary etc

he atlas of common eye disorders unfortunately only consists of black and white tographs

1 the next edition a chapter about the preparation of a scientific paper could haps be added

he book can be warmly recommended to any ophthalmic assistant

M S \orn

n Babel Nicolas Stangos Silvio Korol & Micheling Spiritus Ocular Electrophysiology *A Clinical and Experimental Study of Electroretinogram* Electro Oculogram Visual Evoked Response Georg Thieme Publishers Stuttgart 1977 172 pages 145 Figures 21 Tables Price Da Kr 264 00

the short introduction the authors stress the difficulties which have arisen especially om the clinical view point in registering from a localised zone and in attempting to ain standardization of the methods of examination

The authors have since 1968 developed and standardized a reproducible technique r electrophysiological registration by means of a computer both in normal and in thological cases

The book has four chapters and the clinical examinations of electroretinography RG) electrooculography (EOG) and visual evoked response (VER) are individually scussed in separate chapters The fourth and last chapter deals with experimental vestigations concerning the correlation between morphology and electrophysiology Each of the three clinical chapters on ERG EOG and VER have the same com osition in that they begin with a description of the background for the electrophysio gical registration The authors then describe their own technique and the results btained when examining normal subjects

Furthermore each chapter has a section showing examples of pathological ERG EOG nd VER in various diseases of vascular degenerative and inflammatory nature in he retina, pigment epithelium and the optic nerve This is further illustrated by case eports fluorescein angiograms drawings of the pathological registration and a com

parison of the results which can be obtained in the individual case by ... retinography or electrooculography In the fourth and final chapter ... mental examinations the effect of intravenous or intravitreal injection of ... stances f ex monoiodacetate ferro oxide xylocaine and glycine on the ... potentials is shown By means of electronmicroscopy the nerve or cell ... the toxic substances cause are shown and in this way it is possible to ... origin of the abnormal registrations in clinical electrophysiology

Even though the book deals with an extensive subject – clinical electro... in comparatively few pages it does however manage to be comprehensive ... It is easily read and answers many questions It can be warmly recommended

S F...

Documenta Ophthalmologica Proceedings Series vol 11 XIIIth ISCERG ... sium Kibbutz Ginossar Israel 1975 *Experimental and Clinical* ... (Eds Auerbach & Henkes) 1977 Pp 207 89 Figs 8 Tables. Price Dutch Glds 60 – ISBN 90 6193 151 7

This volume presents basic and clinical studies of amblyopia and other ... the visual system. Research in the field of normal visual physiology is also ... The title subject is dealt with in papers e g on single unit recordings from the ... cortex in cats with induced heterotropias and on photopic spectral sensitivity ... opic and normal eyes The electrophysiological investigation in the presence of ... opacities makes up the topic for another important section of the book ... instance demonstrated that the prognosis of a patient with diabetic ... a vitrectomy is correlated to the magnitude of the pre operative photopic ... Other reports deal with electrophysiological patterns in colour vision defects ... or peripheral retinal degenerations and anomalies of the optic nerve

The last part of the book is devoted to various topics such as the ... stimulus duration on the oscillatory potentials taurine as an inhibitory ... mitter and changes in retinal ganglion cell function during acute elevation of IOP

All papers are in good English but abstracts are omitted in ... booklet has a natural place in the library of clinics and laboratories ... in visual physiology

E.J.K.

Rehak S Krasnov M M & Paterson G D Recent Advances in Glaucoma ... tional Glaucoma Symposium Prague 1976 ... Figs 46 Tables. XIII ... 1977 Cloth DM 78 – US $ 39 00 Berlin Heidelberg New York Springer Verlag Prague Verlag Avicenum ISBN 3 540 0 914 0

In 1976 an international glaucoma symposium was held in Prague organized by ... Czechoslovakian Ophthalmological Society in cooperation with All Union ... mological Society USSR The reports from the meetings are now published ... comprising a little less than fifty papers

ut half of these are studies from Eastern Europe and the rest from the western (one Scandinavian) and Japan. Several of the very well known glaucoma -s contributed to the symposium with papers and discussions
r theoretical part of the symposium concerned the flow of blood and aqueous ir through the eye and the problem of the outflow of aqueous humour
e clinical part comprised glaucoma surgery Laser treatment conservative treat - of open angle glaucoma the problems concerning screening early diagnosis and hypertension After each paper there are references and the discussion is d after each topic. There is a subject index and an author index
the surgical part there is of course a discussion of trabeculectomy and how it ons The indications and selection of operation of primary glaucoma and the lications of antiglaucomatous operations are stated A couple of more unusual dures as goniospasis diathermo trabeculotomy ab externo and the long term s of microdrainage of the anterior chamber using a hydron capillary drain are described The problems of screening for the early diagnosis of primary glaucoma iscussed once again. General screening is not to be advocated, but the advice is netry on each patient who visits the ophthalmologist at least if he is over 35 of age This is closely connected with the next item ocular hypertension and i *to treat it The discussion is very informative and helpful in this difficult* r The new Beta adrenergic and Beta adrenolytic drugs are studied in an Italian r The strange fact is emphasized that both adrenergic beta receptor agonist and receptor antagonist are effective in reducing the intraocular pressure The effect ifferent drugs from the two groups are studied This symposium has covered the non world wide problems of glaucoma in a satisfactory way There are good issions and interesting reports As a small objection, it must be mentioned that are quite a few misprints and some translation errors

Poul Kjer

'rrets Zaralia Diabetic Retinopathy 125 pp 220 Figs Masson Paris Price 150 Fr

author describes different aspects of diabetic retinopathy such as prevalence vidual features major clinical types the natural course medical treatment including itary ablation and diabetic retinopathy and pregnancy
i two chapters the author discusses the results of photocoagulation and vitreous ery in this disease In his opinion which is in agreement with many other statements he literature photocoagulation is of great value in many cases especially those ering from proliferative diabetic retinopathy It is furthermore concluded that ectomy which is a difficult operation with many pitfalls should be reserved only cases where photocoagulation treatment has been ineffective In other words the cations for vitrectomy as a rule begin at the point where those for photocoagula end
he book is instructive and can be recommended for practicing ophthalmologists as l as those dealing with the treatment of diabetic retinopathy

Hans Walther Larsen

Jared M Emery & David Paton Current concepts in Cataract Surgery [illegible] ceedings of the Fourth Biennial Cataract Surgical Congress [illegible] Company St Louis 1976 515 pages numerous photographs figures [illegible] comprehensive subject index (3–4000 references) 8 contributors [illegible]

The book is a report on the 4 Cataract Surgical Congress in 19[illegible] as organiser and chairman It is divided into 162 short chapters which are [illegible] under 9 main sections namely 1) preoperative considerations (incl. [illegible] instrumentation) 2) intracapsular cataract extraction 3) extracapsular cataract extraction 4) special problems in cataract surgery (pre existing glaucoma, [illegible] toplasty etc) 5) intraocular lenses 6) operative complications [illegible] plications 8) correction of aphakia 9) ambulant bilateral or high vol[illegible]

The book contains an abundance of useful information on cataract surgery [illegible] gery of the anterior segment It makes light reading due to its unorthodox [illegible] straight forward presentation In spite of the multifarious contributions from [illegible] *ferent authors the book is quite comprehensible with a* vast subject index and [illegible] sive use of boldface types in formulating the more general and especially [illegible] viewpoints or results The book represents a stimulating and interesting but [illegible] nized kaleidoscope of valuable details concerning methods results, experiences [illegible] opinions from the frontlines of advanced cataract surgery

There is hardly any problem or question large or small concerning cataract [illegible] which is not dealt with in this book The book is therefore considered as being extremely useful didactic and stimulating for any eye surgeon who performs cataract surgery

Eshl G[illegible]

Harold A Stein and Bernard J Slatt Fitting Guide for Hard and Soft Contact Lenses Mosby 1977 Price $ 24 95 (US)

Contrary to other books on contact lenses with the traditional chapters covering history anatomy and physiology of contact lenses this book deals with the [illegible] problems which arise when fitting contact lenses The book is suitable not only [illegible] new beginner but also to the more experienced contact lens fitter

The book comprises four sections together with an appendix

The first section deals with the terminology patient selection, indications and contraindications for contact lenses

The second section is concerned with soft lenses the various types [illegible] and bandage lenses The fitting procedure Cleaning and disinfection The third [illegible] deals with the hard lenses the fitting procedure special problems with [illegible] astigmatism and aphakia

The fourth section contains definite guide lines for the prescribing of both [illegible]

The appendix contains a number of tables such as a keratometer conversion [illegible]

The book is well illustrated with many good drawings and pictures [illegible] comprehensible The book is indispensable for the new beginner and [illegible] good advice for the more experienced fitter The book is to be recommended

[illegible]

~us Jacobsen (Ed.) Handbook of Sensory Physiology vol 9 Development of Sensory Systems Springer Verlag Berlin Heidelberg New York 469 pp 1978 DM 250 DKr ca 578 –

book deals with the development of sensory systems and in particular emphasizes aspects of their structure and function which could not be derived from the study fully developed systems Looking at successive developmental stages of a sensory ~m permits unique insights which would have been much more difficult if not possible to obtain from studying the adult system

he work is intended as a reference for researchers in developmental biology neuro siology and behavioural sciences In the field of ophthalmology it cannot be claimed have a general professional interest but it would seem to be most helpful for those icularly engaged in the study of development aspects of the visual system

he book contains nine chapters of which only two (chapter 4 and 7) are on the ual system Chapter 4 by David Ingle describes the development of visual behavior nonmammalian vertebrates while chapter 7 by Helmut Hirsch and Audie Leventhal its functional modification of the developing visual system of mammals This chapter es an interesting review of experiments on binocular and monocular visual depriva n an actual field in amblyopia research

The book belongs to the monumental Handbook of Sensory Physiology which should known to ophthalmologists and used as a valuable reference work

Niels Ehlers

bert F Schmidt (Ed.) Fundamentals of Sensory Physiology Springer Verlag New York Heidelberg Berlin 286 pp 1978 DM 33 60 DKr ca 135 –

he aim with this introductory text has been to guide the student in biology and the her natural sciences into the realm of sensory physiology Where other areas of urophysiology are involved reference is made to a companion volume Fundamentals Neurophysiology (1978) The two together present an integrated introduction to e neurosciences

Besides a general introduction to the subject and a chapter on the neurophysiology sensory systems the book contains chapters on somatovisceral sensibility vision aring sense of equilibrium taste olfaction thirst and hunger Each chapter is brief nd may be considered as a presentation of the present day status of the field Refer nces to litterature and alternative viewpoints are briefly mentioned

To an ophthalmologist it can be looked upon in two different ways One can focus he chapter on physiology of vision by Grusser and Grusser Cornehls and find an almost too short and concentrated presentation of the subject Alternatively one can ocus on the other chapters and find a readable text well suited to broaden the physio ogical background knowledge

Many of the illustrations are excellent and may be found useful by students as well as teachers

Niels Ehlers

International Symposium on Herpetic Eye Disease

will be held in Freiburg April 12th–14th 1980 Deadline for the subm[illegible] abstracts for the Herpes Symposium will be December 15th 1979
For further inquiries please contact
Dr Rainer Sundmacher Universitatsaugenklinik Killianstr., D [illegible] Fre[illegible] Fed Rep Germany

IV Symposium – Metabolic Eye Disease

The Fourth Symposium of the International Society on Metabolic Eye Disease [illegible] held in Cairo Egypt February 11th to 13th 1980 under the auspices of [illegible] Muhammed Anwar El Sadat The Program will include Nutritional Metab[illegible] Disease Metabolic Cataracts and the Role of Metabolism in Ocular Infect[illegible]
A second session of the Symposium will be held in Jerusalem Israel Februa[illegible] to 21st 1980 to deal with Diabetic Retinopathy Mucopolysaccharidoses a[illegible] Retinal and Corneal Disorders
For inquiries and submission of free papers contact Program Chairman [illegible] Haddad M D 1200 Fifth Avenue New York New York 10029 USA

International Society of Geographic Ophthalmolo[illegible]
V I International Congress

will be held 10–14 September 1979 in Rio de Janeiro Brasil – Offi[illegible] I – Parasitical Eye Diseases II – Corneal Pathology III – Causes of B[illegible] Visual Aids Lecture Workshops Free Papers and Geographic Ophthalmologic[illegible]
For further information contact
Congress Secretaria Av Maracana 377 Rio de Janeiro Brasil

13th Course and Workshop in Clinical Echo Ophthalmo[illegible]

A Scan B Scan and Doppler Echography in intraokular and orbital d[illegible]
Location II Universitats Augenklinik Wien
Time June 26–30 19 9
For further information and registration contact Doz Dr Peter Till
Oberarzt der II Univ Augenklinik Alserstrasse 4 A 1090 Wien, Austria

University Eye Department (Chief T L Thomassen)
Rikshospitalet University of Oslo

CHOROIDAL FLUORESCEIN ANGIOGRAPHY IN RABBIT

BY

AMUND RINGVOLD and ERLING GRØNVOLD OLSEN

The extreme permeability of the choriocapillaris to sodium fluorescein makes angiography with this tracer unsuitable to shew the choroidal vascular bed In order to prevent this profuse leakage the choroidal circulation is visualized with fundus angiography after injection of fluorescein isothiocyanate (FITC) labelled dextrans of different molecular weight 20 000 40 000 70 000 and 150 000 It is shown that the two first dextrans leak out of the choriocapillaris rather rapidly whereas the last two dextrans remain intravascularly long enough to enable good demonstration of choroidal vessels The practical application of these findings is discussed and it is concluded that the dextran with molecular weight 70 000 should be preferred for use in humans

Key words angiography – dextran – fluorescein – rabbit – capillaris – choroidal vessels – permeability

rmal and pathologic retinal circulation as well as abnormal barrier condi ns have been extensively studied with fluorescein angiography and in many es this approach has shed new light on the aetiological factors involved in hthalmic diseases such as central serous retinopathy pigment epithelial tachment and others A similar procedure to visualize the choroidal circula n would certainly also be of great value but so far no method has been devel ed which can demonstrate the choroidal vascular pattern clearly Some in rmation concerning this circulation is obtained both from conventional fluores in angiograms (Hyvarinen et al 1969 Oosterhuis & Boen Tan 1971 Archer & nest 1974 Hayreh 1974) and from infrared fundus angiography after Indo anine green injection (Hill & Young 1975 Craandijk & van Beck 1976

ceived December 27 1978

Flower & Hochheimer 1976) and with choroidal absorption a ~ patent blue V (Sorensen 1977) However the results are impa ed ~ intervening pigment epithelium and in addition, the free diff ~ rescein from the choriocapillaris into the extravascular space ~ the individual choroidal vessels in all but the very early phase of

The present study was started as an attempt to overcome the these problems In order to prevent blurring in the angiograms d ~ t leakage of fluorescein from the fenestrated choriocapillaris we ~ study of choroidal angiography with fluorescein bound to ~ dextran particles

Material and Methods

This investigation includes two adult rabbits (Dutch and Chinchilla sedated with 0 15–0 20 ml fluanisonum(0 1 %) (Hypnorm vet. ~ muscularly about 10 min before angiography The experiment ~ performed on the right eye after maximal mydriasis with cyc chloride(1 %) and phenylephedrine(10 %) drops The fluorescein ~ ministered either as the concentional 10 % sodium fluorescein ~ or as a 20 % solution of fluorescein isothiocyanate(FITC) conj ~ in Ringer(10 ml) (The dextrans were generously provided from ~ Ltd Sweden) The degree of fluorescein substitution of the dextrans one residue per 100–1000 glucose residues (Bellhorn et al 19~) with 10 % FITC solutions (10 ml) therefore gave insufficient vasc ~ (unpublished) Both animals underwent angiography successively ~ particles of four different molecular weights (20 000 40 000 ~ later referred to as FITC 20 FITC 40 FITC 70 and FITC-150 ~ and there was always at least a one week interval between each ~ on every animal Osmolarity estimation of the different solu ~ Osmometer gave the following values (mo m/kg) Ringer 25~ FITC FITC 40 351 FITC 70 337 FITC 150 335 The dye solutions we~ as rapidly as possible through a marginal ear vein but due to ~ increase in viscosity of the solutions with increasing dextran mol ~ horn et al 1977) the injection time was considerably prolon ed (FITC 150) towards the higher molecular weights The first angio~ phase) were taken successively some few minutes after the ~

Fig 1
Choroidal angiography of a rabbit(Dutch) a) sodium fluorescein b) FITC ~ phase One particular choroidal vessel shown in all Figs 1 b–3 b ~

completed whereas the late phase was taken one hour later Zeiss fundus Camera with Robot Motor Recorder and Nikon wide angle lens using Retinapan 45 were used The respective equipments were run with ... and Kodak Tri X film developed in Ilford ID 11

Results

Fundus angiography after injection of 5 ml sodium fluorescein showed in all animals the well known sequence of diffuse choroidal fluorescence with massive extravasation from the choriocapillaris and distinct visualization of retinal vessels (Fig 1 a) In contrast to this after fluorescein labelled dextran injection choroidal vessels appeared in all early phase angiograms ... the FITC 20 tracer allows identification of lager vessels only ... there is decreasing leakage of the tracer with increasing molecular weight of the dextrans (Fig 1 b 2 3) but even FITC 150 leaks out in time giving some blurring of the vascular pattern one hour after the injection. Since slight haziness of the vessel contours are also present in early angiograms after FITC 70 injections (Fig 2 a) one has to base details of the choroidal vascular bed on early angiograms with the FITC 150 tracer Fig ... *For comparison* it was intended to visualize retinal and choroidal vessels in *the same* field and accordingly most angiograms show the ... fundus A part of the fundus just below the papilla is seen in Fig ... arrows in these pictures indicate one particular choroidal vessel present in angiograms from Figs 1 b–3 b In this region striking zone ... towards which choroidal vessels from both sides taper off Further in Fig 3 a it is illustrated that the larger vessels in this particular parellel divide dichotomously and give off smaller branches which apparently are transformed rather abruptly into the non visualized meshwork The coarse grained appearance of the single choroidal (Fig 3 a) may perhaps represent numerous smaller connections to the capillary meshwork or it may have been brought about by the granulation of the pigment epithelium The smallest choroidal vessels visualized with this ... are of the same diameter as the retinal vessels in the peripapillary ... (Figs 2 a 3 a) i e about 100 μm across (Prince 1964) A distinction between arteries and veins seems impossible in the angiograms because ...

Fig 2

Choroidal angiography of a rabbit(Dutch) FITC 70 early(a) and late (b) phase ... hour after injection)

a

b

tly increasing intravasal fluorescein concentration had become high enough ake photographs all the vessels were filled with tracer substance Princip the same findings were obtained in the Dutch(black and white) and the nchilla(brown) rabbits although the choroidal vessels appeared more granu d *in angiograms from the brown rabbits It should be stressed that both* nals were in good condition throughout the experiments and toxic effects e neither noted during the injections nor in the post experimental period

Discussion

seen in the present illustrations the choroidal vessels appear quite clearly r injection of the larger dextran molecules This indicates that although iously there is some blurring of the choroidal circulation in angiograms due he intervening pigment epithelium the extravasation of fluorescein seems be the main reason why ordinary fundus angiography fails to show details the choroidal vascular bed However on the whole the angiograms show oroidal vessels more indistinctly in the brown than in the black and white bit and whether *this reflects real differences in the choroidea or refers to* tors in the pigment epithelium cannot be determined from our results

It has been demonstrated in many species that the wall of the choriocapillaris penetrated by numerous circular fenestration (about 80 nm across) closed a diaphragm (Rohen 1964 Hogan et al 1971) These capillaris are highly rmeable to large molecules such as myoglobin(mol wt 17 000 4 nm dia ter) whereas the still larger molecules albumin and gammaglobin(7nm and nm diameter spheres respectively) are drained at a lower rate (Bill 1968) is impaired or controlled leakage of high molecular serum components may er to permeability properties of the diaphragms As expected from these data e ability to prevent leakage of fluorescein from the choriocapillaris depends on e molecular size/weight of the dextran particles to which it is conjugated but nce even globulins pass out in time in substantial amounts it was not clear what ze of dextran particles would be sufficient to prevent the choroidal flush by fun is fluorescein angiography We therefore used four different dextran tracers nol wt 20 000 40 000 70 000 and 150 000) with the diameter 6 4 nm 9 nm 11 8 m, and 17 4 nm respectively (Pharmacia Fine Chemicals technical data bulletin) oping that leakage of the smaller ones would be slow enough to show choroidal

Fig 3

horoidal angiography of a rabbit(Dutch) FITC 150 early(a) and late(b) phase (one hour after injection)

vessels at least in the early phase of angiography It turned out th FITC 40 angiography revealed definite choroidal vessels but unfor... marked blurring already in the initial phase This probably ... the prolonged injection time caused by the high viscosity of the ... rather small dextrans penetrate the choriocapillaris in sub... before the intravascular marker concentration is high enough ... monstration of the choroidal vessels angiographically Accord... visualization of the choroidal vessels was first achieved after ... FITC 70 and 150 The leakage of the FITC 150 was so slow th... vessels appear rather distinctly even in the late angiograms i e ... the tracer injection

In conclusion fundus angiography after injection of fluoresce... dextrans is a new tool to study not only retinal disease (Rabkin et al ...) but also choroidal circulation and pathology in vivo Since some ... red cell aggregation occurs after injection of dextrans with higher ... weight such as FITC 150 (Stalker 1967) it seems that FITC 40 is ... attractive tracer for use in humans This dextran particle size has ... used in Macrodex(Pharmacia) for clinical purpose although not as a ... labelled compound

References

Archer D B & Ernest J T (1974) Angiographic studies of the chor...dal ... *Int ophthal Clin 14* 89–103

Bellhorn M B Bellhorn R W & Poll D S (1977) Permeability of fluoresce... dextrans in fundus fluorescein angiography of rats and birds *Exp Eye R...* 59 ... 60 ...

Bill A (1968) Capillary permeability to and extravascular dynamics of ... albumin and gammaglobin in the uvea *Acta Physiol scand* ...

Craandijk A & van Beek C A (1976) Indocyanine green fluorescence a... the choroid *Brit J Ophthal 60* 37...–...

Flower R W & Hochheimer B F (1976) Indocyanine green dye fluores... infrared absorption choroidal angiography performed simultaneously with ... angiography *The Johns Hopkins med J 138* 33–4...

Hayreh S S (1974) Recent advances in fluorescein fundus angiography *Brit J O...* ... 391–41...

Hill D W & Young S (1973) Infrared angiography of the cat fundus ... *Ophthal (Chicago) 93* 131–133

Hogan M J Alvarado J A & Weddell J E (1971) *Histology of the h...* B Saunders Company Philadelphia London Toronto

Hyvarinen L Maumenee A E George T & Weinstein G W (19...) F... angiography of the choriocapillaris *Amer J Ophthal 6...*

Oosterhuis J A & Boen Tan T N (1971) Choroidal fluorescence in the ... *Ophthalmologica 162* 246–260

ce J H (1964) *The rabbit in eye research* C C Thomas Publisher Springfield
inois U S A
-on M D Bellhorn M B & Bellhorn R W (1977) Selected molecular weight
xtrans for in vivo permeability studies of rat retinal vascular disease *Exp Eye*
24 607–612
-n J W (1964) Das Auge und seine Hilfsorgane In *Handbuch der mikroskopischen*
natomie des Menschen Bd III/4 Springer Verlag Berlin Gottingen Heidelberg
ew York
ker A L (1967) The microcirculatory effects of dextran *J Path Bact 93* 191–201
nsen P N (1977) Choroidal absorption angiography with patent blue V *Acta*
hthal (Kbh) 55 471–477

hors addresses
Amund Ringvold
iversity Eye Department
shospitalet
o 1
rway

Erl n Grønvold Olsen
iversity Eye Department
tshospitalet
lo 1
rway

Department of Ophthalmology (Heads E Goldschmidt S Fors... University Hospital Odense Denmark

MACULAR OEDEMA IN THE REATTACHED RETINA

BY

PREBEN GILBERT NIELSEN

A material consisting of 24 consecutive patients has been ... spectively after a successful retinal detachment opera... oedema was demonstrated by fluorescein angiography in ... (3?%) and was found to be the most frequent cause of poor re... of visual function Fluorescein angiography is essential for the ... of macular oedema which may be difficult to demon... methods The present investigation indicated that subretinal d... be a factor in the development of a post detachment syn... macular oedema

Key words macular oedema – retinal detachment – fluorescein an... graphy – subretinal drainage

Macular oedema is a potential complication during the postoperat... following ocular surgery In this respect Cass & Norton (19..) dem... using fluorescein angiography the retino vascular origin of cys... oedema in the postcataract extraction syndrome

Macular oedema has long been known to be a postoperative com... retinal detachment also in phakic patients

Poor recovery of visual function is a not infrequent occurrence af... ful retinal detachment surgery with an apparently uncomplicated po... course A deterioration in visual acuity can occasionally be observ... satisfactory initial results Until recently fluorescein angiograph... the macula in the reattached retina have only been referred to sp...

Received December 28 19..

literature Cleary & Leaver (1978) published a systematic study of the ached macula using fluorescein angiography

ie present investigation was commenced in order to study a material of nts with retinal reattachment using fluorescein angiography Particular hasis being attached to the occurrence of macular oedema and possible rs predisposing to this condition

Material and Methods

tal of 89 patients were subjected to operation for retinal detachment during riod of two years (1976–78) at the Ophthalmological Clinic of the Odense versity Hospital The following were excluded from the material in order liminate abnormalities of complex origin Those requiring more than one ation for reattachment of the retina cases with aphakia detachment pre ed to be of *traumatic origin excessive myopia* (≥ -8D) *pre existing* ular disease and cases with opacities in the ocular media The material reduced in this manner to 28 patients Two failed to attend the follow up mination and two had died Thus there remained a total of 24 patients have been examined at intervals varying from 3 to 26 months post atively The visual acuity of each patient was determined The fundus of eye was examined by direct and indirect ophthalmoscopy and the condi of the macula evaluated biomicroscopically Colour photographs were n and fluorescein angiography carried out on all patients

he duration of the retinal detachment varied from one day to in a single ent three months The average duration of retinal detachment was 12 8 s and the macula was detached in 14 of the 24 patients Their ages ranged n 13 years to 78 years with an average age of 63 years

hod of operation

the patients were subjected to a scleral buckling procedure Local silicone lant was *employed in 13 cases An encircling procedure alone or combined* h local explant *was used on the remaining patients* In 11 cases cryopexia diathermia was employed through full thickness sclera In the remaining 13 es no cryopexia or diathermia was used

Subretinal drainage was carried out in 22 of the 24 cases This was estimated being excessive in seven patients moderate in 12 and in three patients only ht drainage was necessary

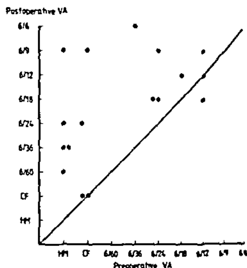


Fig 1

Scattergram showing changes in visual acuity (VA) in 24 eyes after re
(HM = hand movements CF = counting fin

Results

The changes in visual acuity are shown in Fig 1 The visual ac
in one patient was unchanged in three and improved in the rema
(20) at the time of follow up examination and as compared to t
operatively

Macular oedema was found in a total of nine patients (35 The c
examination revealed macular oedema in five patients only fl
graphy verified the presence of the condition in all five patients a
tion demonstrated macular oedema in a further four cases where n
signs of this abnormality could be observed during the clinical exa
six of these nine cases the angiograms showed typical cystoid
with a central dark stellate figure surrounded by fluorescein
intraretinal cystoid spaces In the remaining three patients the oedema
amorphic type

The presence of macular oedema was synonymous with a visual a
6/18 or less with the exception of one patient where the visual a
Fig 4 shows the visual acuity of the patients with macular oedema a
to those without this complication

A reduction in visual acuity was observed in four patien at
follow up examina ion when compared to the visual acuity on disch

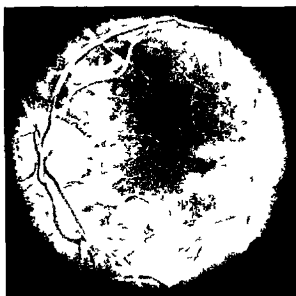


Fig 2
orescein angiogram late phase (11 min) showing macular oedema at three months

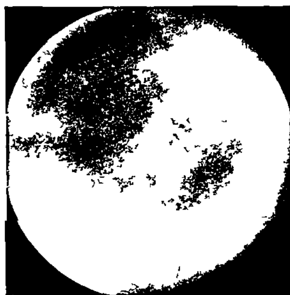


Fig 3
luorescein angiogram late phase (30 min) showing typical cystoid macular oedema at 13 months

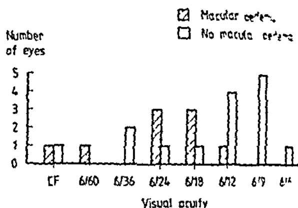


Fig 4
Histogram showing visual acuity postoperatively in nine eyes w[illegible] macular oedema

10 postoperatively) It was possible to demonstrate macular oedema a[illegible] of the follow up examination in three of these four patients w[illegible] a reduction in visual acuity

Macular oedema could be demonstrated in five of the seven cases [illegible] excessive subretinal drainage while with moderate drainage [illegible] observed in four of 12 cases No oedema at the posterior pole could be [illegible] any of the five cases where there had been none or only slight d[illegible]

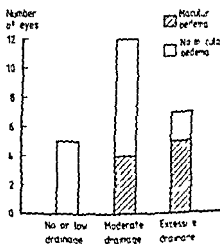


Fig 5
Histogram showing number of eyes with and without macular oedema w[illegible] of subretinal drainage

Discussion

-ular oedema has been demonstrated in 35 % of the patients in the present -stigation by means of fluorescein angiography after an otherwise un -plicated operation for retinal detachment An attempt has been made to -lude from the material other possible cases of macular oedema of non -chment origin In a prospective study of a similar material where fluorescein -iography was systematically carried out Cleary & Leaver (1978) found -ular oedema in 17 of 66 cases (25 7 %) Bernardczykowa (1975) de -strated biomicroscopically macular oedema in nine of 30 examined -ents (30 %) In the present material subretinal drainage was carried out approximately 92 % of the cases In comparison subretinal drainage was d in only approximately 40 % of the cases in the material of Cleary & -ver (1978) The operative technique employing subretinal drainage in -lition to scleral buckling inevitably involves considerable manipulation and -ormation of an extremely soft eye Changes in the vitreo retinal interface -h vitreous detachment at the posterior pole is a likely consequence and -reo retinal traction a natural result via the normally existing vitreo macular -erence (Grignolo 1952) The development of a post detachment syndrome -h macular oedema can hardly be ascribed to any single factor In the present -estigation subretinal drainage appears to have played a role An actual -nparison of the drainage technique with the non drainage technique has -wever not been possible in the present study where all but two of the -tients were subjected to drainage to a greater or lesser extent The material -s been sub divided according to the estimated extent of the subretinal -ainage and this revealed an accumulation of cases with macular oedema in - group with excessive drainage (Fig 5) In the material of Cleary & Leaver -978) 10 of 27 eyes with drainage showed macular oedema (37 %) as -mpaired with seven of 39 eyes without drainage (18 %) This difference is -tistically non significant with $0\ 05 < p < 0\ 1$ (χ^2 method)

No correlation could be demonstrated between macular oedema and age of -e patient refraction the duration of the detachment or preoperative macular -tachment There is no evidence in the results indicating that use of cryopexia -athermia or local explant contra encircling procedures are factors of im -ortance with regard to the development of macular oedema

Regarding the visual prognosis in the nine patients with macular oedema -is must be considered poor in at least five cases In the e five cases the -uration of the macular oedema was more than one year (observationtime -5–26 months) The observationtime in the remaining four cases was less than -ne year and the final visual outcome therefore still uncertain Cleary & Leaver -1978) found *persistent macular oedema in seven out of a total of 17 cases*

Table I

Macular oedema in relation to subretinal drainage

	+ macular oedema	– macular oe[illegible]
Present material		
+ subretinal drainage	9	13
– subretinal drainage	0	[illegible]
	9 (3[illegible] %)	[illegible]

	+ macular oedema	– macular oede[illegible]
Cleary & Leaver (19[illegible]5)		
+ subretinal drainage	10 (3[illegible] %)	1[illegible]
– subretinal drainage	7 (18 %)	3[illegible]
	1[illegible] ([illegible] %)	49

It has been found in the present investigation that macular oedema was [illegible] most frequent cause of poor recovery of visual function Persistently re[illegible] vision could however not be ascribed to demonstrable macular abnormalities in all of the cases Two patients with normal macular appearance b[illegible] clinically and angiographically obtained maximal visual acuity of 6/[illegible] [illegible] 6 12 respectively In these two cases the reduction in visual acuity must pr[illegible] sumably be ascribed to failure of retinal receptor regeneration. It is kn[illegible] from experimental studies that retinal detachment is rapidly followed b[illegible] degeneration of the retinal receptor cells and that reattachment is follo[illegible] by regeneration (Kroll & Machemer 1969) Previous assumptions of poor re[illegible] receptor regeneration as a relatively frequent cause of severely reduced [illegible] (Chisholm et al 1975 Gundry & Davies 1974) could not be confirmed in th[illegible] present study where macular abnormalities could be demonstrated in th[illegible] great majority of patients with severely reduced vision However incomplete re[illegible] generation of the retinal receptors could be considered as the explanation [illegible] why only one patient obtained a visual acuity of 6/6

It may be concluded that macular oedema is a frequent complication a[illegible] must be suspected in cases of poor recovery of visual function after re[illegible]

t of the retina or where visual acuity suddenly worsens during the post atıve period Fluorescein angiography is an essential part of the diagnosis iacular oedema

References

J D M & Norton E W D (1966) Cystoid macular oedema and papilloedema llowing cataract extraction *Arch Ophthal., Chicago* 76 646–661
ardczykowa A (1975) Maculopathy as consequence of retinal detachment. *Bull cad pol sci biol* 23 839–845
ry P E & Leaver P K (1978) Macular abnormalities in the reattached retina *nt J Ophthal* 6? 595–603
molo A (195?) Fibrous components of the vitreous body *Arch Ophthal Chicago* · 160
dry M F & Davies E W G (19 4) Recovery of visual acuity after retinal detach ent surgery *Amer J Ophthal* 7? 310–314
holm I A McClure E & Foulds W S (19 5) Functional recovery of the retina ter retina detachment *Trans ophthal Soc U K* 95 16 –1 _
ll A & Machemer R (1969) Experimental retinal detachment and re attachment the owl monkey *Amer J Ophthal* 68 58–

hors address
ilbert Nielsen
t. of Ophthalmology
ense Sygehus
0 Odense
nmark.

Department of Ophthalmology (Head B Tengroth)
Karolinska Hospital Stockholm

VITRECTOMY AFTER INTRACAPSULAR CATARACT EXTRACTI[illegible] IN DIABETIC SEQUELAE

BY

PEEP ALGVERE

Pars plana vitrectomy was performed on 40 diabetic eyes with opacified vitreous due to preretinal haemorrhages and fibrovascular proliferations Cataractous lenses were present in all cases and were removed by intra capsular extractions 4–8 weeks prior to vitrectomy Preoperative visual acuity ranged from light projection to 1/60
Postoperatively vision improved in 26 (65 %) eyes remained unchanged in 5 (12.5 %) and deteriorated in 9 (22.5 %) during a follow up time of 5–15 months (mean 8 months) In 19 (48 %) eyes a visual acuity ranging from 0.1–0.9 was achieved In 31 eyes that improved or remained unchanged iris neovascularization developed in 2 (6 %) and longstanding increase of intraocular pressure in 9 (29 %) Visual failures were mainly due to incomplete removal of preretinal proliferations and retinal detachment and neovascular glaucoma developed in 5 of the 9 eyes
In 30 (75 %) eyes the anatomical status at the vitreo retinal junction improved considerably when the preretinal tissue causing retinal traction was excised and the proliferative disease reverted into a background retinopathy

Key words vitrectomy pars plana – diabetes mellitus – cataract extraction – visual improvement – complications – retinal detachment

Since the introduction of special instruments (Machemer et al 19[illegible] Kloti 19[illegible] Douvas 1975 O Malley & Heintz 1975 and others) vitrectomy via pars pl[illegible] has rapidly become an accepted surgical procedure The most common ind[illegible] tions for vitrectomy so far have been the diabetic sequelae associated [illegible] vitreous opacification and preretinal fibrovascular proliferations In such [illegible]

Received December 6 1978

e is a high incidence of lens opacities which spontaneously or following ectomy often progress to dense cataracts requiring lens removal (Faulborn il 1978) Accordingly lensectomy *via* pars plana in the same session as vitrectomy has been widely practiced (Machemer 1975) However hard n nuclei are difficult to remove with any of the vitrectomy instruments now ilable making ultrasonic fragmentation of the lens a necessity Although ical clarity is achieved the lens tissue cannot be entirely removed by ectomy and the lenticular remnants tend to aggravate the postoperative ammation

t has been suggested that (even in connection with vitrectomy) sclerotic ses be extracted intracapsularly with consequent complete removal of all s tissue (Tolentino et al 1977) However performed at the same time as rectomy intracapsular lens extraction may be a more traumatizing procedure n pars plana lensectomy

n the present study vitrectomy was performed on diabetic eyes with long nding vitreous haemorrhages and peretinal fibrovascular proliferations ociated with cataractous lenses The lenses were removed by intracapsular ractions In an attempt to minimize the surgical trauma a two step procedure s adopted the vitrectomy was performed 4–8 weeks after the cataract traction and not before the inflammation following lens surgery had subsided

Materials and Methods

tween April 1977 and March 1978 cataract extractions and pars plana trectomies were performed on 40 patients (18 males 22 females) ranging in e from 30–72 years The patients had been known diabetics for 7 to 47 years iean 29 years) All 40 eyes had proliferative diabetic retinopathies and treous haemorrhages which lead to longstanding vitreous opacification loss of nbulatory vision was recorded between 1 and 12 years (mean 3 1 years) prior n operation

Routine ophthalmologic examination included biomicroscopy with the 3 irror lens and ultrasonography using A scan and B scan according to Cole ian (Algvere et al 1978) Vitreous membranes and preretinal or prepapillary roliferations were observed in all eyes Localized retinal detachments were resent in 13 eyes (33 % of the cases)

The following criteria were used for selecting patients Visual acuity ranged rom perception and accurate projection of light to finger counting at 1 m 1/60) Iris neovascularization was considered a contraindication for vitrectomy Patients with dilated iridial vessels but without glaucoma were not refused

surgery *Large retinal detachments (covering at least* one quadrant of the fundus) associated with preretinal proliferations were not operated on. Electrophysiologic examinations were done in selected cases Electroretinograms (ERG) were recorded in response to strong light stimuli (6 log units above the dark adapted b wave threshold) an eye with an extinguished ERG was considered to have a poor postoperative visual prognosis and was not vitrectomized Visually evoked response (VER) was elicited by flickering light of [illegible] positive results indicating macular function definitely encouraged surgery in dubious cases

Extraction of the opacified lens In this series all eyes had lens opacities and sclerosis or dense cataracts respectively An intracapsular cataract extraction was performed using a standard procedure a corneo scleral (limbal) incision about 160 under a conjunctival flap preplaced corneo scleral sutures a sector shaped iridectomy cryoextraction of the lens Enzymatic zonulolysis was [illegible] for patients under 40 years of age The corneo scleral incision was closed with 10–12 interrupted 8-0 silk sutures Prophylactic cryo treatment was [illegible] over the pars plana and ora serrata in the region where the sclerotomy for later vitrectomy was planned

Vitrectomy Klott s vitreous stripper and endodiathermy unit were used (O Instruments St Gallen Switzerland) *Both instruments are* equipped [illegible] fiber optic sleeves for intraocular illumination and can be inserted into the vitreous through the same sclerotomy since their outer diameters are identical

Under a conjunctival flap a sclerotomy was made 4 5 mm posterior and parallel to the limbus usually in the upper temporal quadrant Weak diathermy was applied to the pars plana in the sclerotomy bed A perforation [illegible] placed inside an infusion cannula was inserted through the pars plana into the vitreous The cannula was sutured to the sclerotomy edges The [illegible] was withdrawn and an infusion of Ringer solution connected to the [illegible] (and vitreous compartment) The intraocular pressure was then determined by the height of the infusion column above the eye

Surgery was performed under a motorized Zeiss (OPMI 6) microscope equipped with X Y coupling (Zeiss Oberkocken W Germany) A [illegible] light contact lens was placed loosely on the cornea abrasion of the corneal epithelium was hardly ever necessary

The aim of surgery was an almost complete removal of the vitreous, leaving only the vitreous base and a thin cortical layer (when that layer was attached to the retina) The fibrovascular proliferations were cut as close to the retina and optic disc as possible The resection was very ofte combined with [illegible] endodiathermy of preretinal vessels

olo ic examination of the material obtained from the vitreous space was ied out in 12 cases The aspirated suspension was centrifugated the pre ated material fixed in formaline mounted in paraffin sectioned and ied with haematoxyline eosine or van Gieson dye

ow up examinations were performed at regular intervals (monthly for the 3 months) and the follow up time ranged from 5–18 months (mean 8 ths) comprising all patients in the study

Results

intracapsular cataract extraction was well tolerated by the diseased eyes the postoperative inflammation as a rule subsided within four weeks An ntended extracapsular operation occurred in four eyes leading to a con rably more severe but still transient postoperative inflammation During



Fig 1

icrograph of proliferating tissue excised from the vitreous space showing fibro ascular proliferations with newly formed vessels and van Gieson staining collagen (original magnification 500 ×)



Fig 2

Fundus photograph 14 months after vitrectomy and resection of fibrovascular pr-tions of 6 year old male who had been socially blind for 3 years Duration of di: 45 years *There is a remaining stalk of fibrous tissue in front of the op t* Postoperative visual acuity 0 7 (aphakic correction)

the first postoperative week anterior chamber haemorrhage was record d several eyes Large postoperative haemorrhages occurred in five eyes w which had very advanced proliferations in the vitreous space and later show a poor visual prognosis

The opacified vitreous was successfully removed from the post rior p of the fundus The excision of preretinal membranes and fibrovascular p-liferations was much more difficult since they were firmly a ached to the retina or optic nerve head Histologic examination of the material o from the vitreous space showed various blood degradation products s d red cells and fibrin and proliferating fibrovascular tissue Fibrous ti sue w seen surrounding the newly formed vessels and contained van Gieson s collagen (Fig 1)

The preretinal tissue was successfully excised from 30 eyes and the op al transparency of the vitreous space restored (Fig 2) The anatom cal situ at the vitreo retinal junction was considerably improved when th

nl traction was eliminated (Figs 3 and 4) Eyes in which such traction not eliminated at surgery and vitreo retinal attachment persisted later loped severe complications (see Visual failures)

e recovery of vision occurred mainly during the first two months after ctomy but minor improvement was observed up to six months At follow xaminations (on the average eight months after vitrectomy) an improve of vision was present in 26 (65 %) eyes A visual acuity ranging from 0 9 was achieved in 19 (48 %) operated eyes (Table I) Seven eyes gained nor visual increase (finger counting 2–5 m) these patients enjoyed some ulatory vision that facilitated their social but not their professional rities

lthough the optical transparency was restored in most eyes normal visual ties were not achieved Various sequelae of retinopathy were present and cially macular degenerations were commonly seen after vitrectomy in y cases these were associated with thin epiretinal proliferations that could be excised In the majority of eyes occlusions of retinal vessels were



Fig 3

ndus photograph 6 months after vitrectomy showing persisting vitreo retinal traction nds to a retinal fold which progressed to a detachment (arrows) Male aged 35 years socially blind for 2 years Postoperative visual acuity 0 2 (aphakic correction)



Fig 2

Fundus photograph 14 months after vitrectomy and resection of fibrovascular proliferations of 6[illegible] year old male who had been socially blind for 3 years Duration of diabetes 45 years There is a remaining stalk of fibrous tissue in front of the op[illegible] Postoperative visual acuity 0 7 (aphakic correction)

the first postoperative week anterior chamber haemorrhage was recorded in several eyes Large postoperative haemorrhages occurred in five eyes fo[illegible] which had very advanced proliferations in the vitreous space and la[illegible] a poor visual prognosis

The opacified vitreous was successfully removed from the posterior [illegible] of the fundus The excision of preretinal membranes and fibrovascular proliferations was much more difficult since they were firmly attached to retina or optic nerve head Histologic examination of the material [illegible] from the vitreous space showed various blood degradation products [illegible] red cells and fibrin and proliferating fibrovascular tissue. Fibrous t[illegible] seen surrounding the newly formed vessels and contained van Gieson s[illegible] collagen (Fig 1)

The preretinal tissue was successfully excised from 30 eyes and th[illegible] transparency of the vitreous space restored (Fig 2) The anatomical s[illegible] at the vitreo retinal junction was considerably improved when the [illegible]

Table II
Primary causes of visual failure (9 eyes)

	Number of eyes
Incomplete removal of fibrovascular proliferations	3
Persisting retinal detachment	4
Profuse postoperative haemorrhage	1
Neovascular glaucoma	1

-ountered and circulatory insuffiency was probably the major cause of the -atisfactory macular function

ual failures In nine eyes a permanent decline of vision and a general terioration of the ocular disease occurred following vitrectomy (Table II) iese cases had extensive preretinal fibrovascular proliferations which were fficult to remove In three eyes only part of the huge vascular network in -e vitreous space could be resected and traumatized fibrovascular tissue had be left in the vitreous Postoperatively retinal detachment and neovascular aucoma developed

In five eyes retinal detachments were present at surgery three of which iowed retinal breaks in the posterior pole In one eye an iatrogeneous retinal ole was produced when excision of a traction membrane was attempted cleral buckling was performed in two eyes but was technically difficult and -mained unsuccessful Since the retinal breaks were located close to the optic isc(s) in areas of fibrovascular membranes that could not be removed scleral uckling was abandoned in the remainder of the eyes

One eye developed a profuse postoperative haemorrhage that did not resorb n vitreous lavage was performed but blood soon refilled the vitreous space ind retinal datachment ensued

Persisting retinal detachment was the major cause of visual failure after vitrectomy and was often associated with glaucoma and iris neovascularization (Table III)

Increased intraocular pressure (IOP) A transient increase of IOP (30 mmHg or more) was common during the first postoperative weeks and was mainly of erythroclastic origin A longlasting increase of IOP (for two months or more)

Table III

Complications observed after vitrectomy in various visual acuity groups of eyes

Visual acuity	Number of eyes	Retinal detachment	Glaucoma		Iris neo-vascular		Ph[illegible] bulbi
Amaurosis – light percept	9	8 (89 %)	5 (56 %)		5 (56 %)		[illegible]
Light projection – 1/60	5	1	2	(29 %)	1	(6 %)	
2/60–5/60	7		3				
0 1–0 9	19		4		1*		

* extracapsular lens extraction

was seen in 14 of 40 eyes but in only 9 of the 31 eyes that either impro[illegible] or remained at the preoperative visual acuity following vitrectomy (Table I[illegible] These eyes required medication to normalize the IOP It must be pointed [illegible] however that the non vitrectomized fellow eyes of four of these patients [illegible] a long history of glaucoma

Iris neovascularization Biomicroscopy (magnification 26 x) and gonioscopy [illegible] closed a transient postoperative dilatation of iris vessels in several eyes pa[illegible] cularly in those with new intraocular haemorrhages This phenomenon [illegible] quite marked in eyes that showed iris vasodilatation preoperatively P[illegible] operative development of iris neovascularization was seen in 7 of 40 eyes in only 2 of 31 eyes without retinal detachment and was associated [illegible] recurrent intraocular haemorrhages and long-lasting increase of IOP (Table [illegible]

Discussion

The prerequisite for a good surgical result is a technically satisfactory v[illegible] tomy that enables adequate removal of the opacified vitreous and the pre[illegible] fibrovascular proliferations The two major goals of vitrectomy can th[illegible] fulfilled the restoration of the optical transparency of the vitreous spac[illegible] the elimination of vitreo retinal traction

These goals can only be achieved if the visualization of the vitreous is satisfactory during surgery In the present series all eyes showe[illegible] opacities or cataracts and therefore a two step procedure was adopt[illegible]

ng an intracapsular lens extraction and – 4 to 8 weeks later – a vitrectomy vitrectomy and especially the excision of preretinal proliferations is nplished much easier in aphakic eyes with sector shaped iridectomies than ıakic ones In phakic eyes visualization of the operation field may be ıred by such factors as lens opacities incompletely dilated pupil (e g with synechiae) or blood in the retrolental space In addition the two step re enabled cryo treatment of the pars plana and ora serrata during the operation stabilizing the tissues around the sclerotomy for the later ctomy Accordingly no retinal dialysis was observed in this series of ations

asoproliferative substances are assumed to be released from a hypoxic ıa (Ashton 1961) and may cause iris neovascularization These agents must use into the anterior segment and such diffusion is facilitated when barriers ı as the lens and vitreous are removed It can thus be argued that following ep wise removal of these barriers the vasoproliferative substances probably h the anterior segment gradually On the other hand if the lens is clear u h to be left in the eye anterior uveitis glaucoma and iris rubeosis are likely to be induced by the vitrectomy

n the present series there was an improvement of vision in 65 % of the s. and in 48 % a visual acuity of 0 1 or more was achieved Following rectomy for diabetic vitreous complications Mandelcorn et al (1976) found overall success rate of 53 % and 43 % of the eyes gained 6/60 or more visual acuity Their recent report on 5 year follow up examinations con med that these surgical results were quite stable and longlasting (Blankenship Machemer 1978) Peyman et al (1976) reported an improvement of visual uity in 71 % of eyes operated on at least six months after the onset of reous haemorrhage Michels (1978) observed visual improvement in 65 % the eyes and in 46 % af final vision of 20/200 or better was achieved

It is quite obvious that the rate of visual success is dependent on the extent preretinal proliferations and the presence or absence of retinal detachment eoperatively Dense vitreous haemorrhages without fibrovascular prolifera ons generally have a good prognosis following vitrectomy

In the pre ent series fibrovascular proliferations were found in all eyes nce excised such proliferations were permanently eliminated and growth f new tissue into the vitreous space never occurred This is a remarkable henomenon showing the value of vitrectomy in diabetic proliferative retino athy The present study confirms similar observations made by others (Michels 9 8 Blankenship & Machemer 1978) The vitrectomy thus changes a retino athy with preretinal membranes and fibrovascular proliferations into a back round retinopathy The progressive course of the vitreo retinal traction is

interrupted Thus complications of retinal traction such as detachment and degeneration of the retina and haemorrhages from vitreo retinal adhesions retinal vessels are alleviated or prevented In addition the toxic blood degradation products are removed from the eye If necessary the background retinopathy can then be photocoagulated

Vitreous haemorrhages do occur following vitrectomy Postoperatively many patients reported transient blurring of vision which was caused by recurrent haemorrhages However in all eyes without retinal detachment (except with profuse haemorrhages) vitreous blood was spontaneously resorbed within a week leaving no opacities This favourable course can probably be explained by the fact that in aphakic eyes vitreous blood easily gains access to the anterior chamber and red cells pass out through the chamber angle In earlier experiments intravitreally injected red cells did gain access to the anterior chamber in aphakic but not in phakic eyes (Algvere & Bill 1979)

The surgical failures in the present series were mainly due to incomplete removal of preretinal proliferations and to retinal detachments As previously reported (Algvere 1978) vitreo retinal traction bands persisting after vitrectomy will contract weeks or months later and lead to retinal detachment These eyes show the most severe complications including iris rubeosis and neovascular glaucoma

In this series the overall incidence of postoperative iris neovascularization was 18 % However if the eyes lost to retinal detachment are subtracted from this figure only 6 % developed iris neovascularization Iris rubeosis following vitrectomy was reported to occur in 26 % (Mandelcorn et al 1976) to 40 % of eyes (Michels 1978) most of which had undergone a pars plana lensectomy in the same session as vitrectomy The two step procedure with intracapsular lens removal and later vitrectomy seems to be preferable in respect to the postoperative development of iris rubeosis

Increased intraocular pressure following vitrectomy in diabetics is common and was reported to occur in 9 % (Gitter & Cohen 1976) to 49 % of eyes (Weinberg et al 1976) In the present series elevation of IOP for at least two months was encountered in 35 % of eyes but if those lost to retinal detachment are excluded the figure drops to 29 % Neovascular glaucoma dominated in the group of surgical failures In the remainder of the cases neovascular glaucoma was present in association with persisting retinal detachment (one eye) and following extracapsular cataract extraction (one eye) Usually other types of glaucoma were seen (erythroclastic or haemolytic cortison glaucoma simplex glaucoma) some of which would probably have developed without vitrectomy since they were present in the non operated fellow eyes

Addendum

follow up periods of 6–26 months (median 16 months) 55 eyes operated ith these methods showed the following results The visual acuity had orated in 11(20 %) eyes remained unchanged in 8(14 5 %) and improved (65 5 %) In 29(53 %) eyes a visual acuity ranging from 0 1 to 0 9 was red In the groups of improved or unchanged vision glaucoma was seen ?3 %) eyes and iris neovascularization in 2(5 %)

us the results were generally stable and lasted during the longer follow riods

References

ere P (1978) Indications for pars plana vitrectomy The present value and limita ns of vitreous surgery *J Swedish Med Ass 75* 2270–2274

ere P Epstein D Jerneld B & Linde C J (1978) B scan ultrasonography in ular diagnostics *J Swedish Med Ass 75* 4?2–475

ere P & Bill A (1979) Drainage of microspheres and red cells from the vitreous aphakic and phakic eyes Arch Ophthal (Chicago) (in press)

on N (1961) Neovascularization in ocular disease *Trans ophthal Soc U K 81* 5–161

ikenship G W & Machemer R (1978) Pars plana vitrectomy for the management severe diabetic retinopathy An analysis of results five years following surgery *rans amer Acad Ophthal Otolaryng 85* 553–559

vas N G (1975) Microsurgial roto extractor instrument for vitrectomy *Mod Probl phthal 15* 253–?60 Karger Basel

lborn J Conway B P & Machemer R (1978) Surgical complications of pars plana itreous surgery *Trans amer Acad Ophthal Otolaryng 85* 116–125

er K A & Cohen G (1976) Complications of vitrectomy In Gitter K A Ed *urrent Concepts of the Vitreous Including Vitrectomy* p 253–26? The C V Mosby ompany Saint Louis

ti R. (1974) Vitrektomie II Chirurgische Technik mit dem Vitreous Stripper *llbrecht v Graefes Arch klin exp Ophthal 189* 1?5–135

chemer R Parel J M & Buettner H (1972) A new concept for vitreous surgery I nstrumentation *Amer J Ophthal 73* 1–7

chemer R (1975) Vitrectomy *A Pars Plana Approach* (Current ophthalmology monographs) p 86–90 Grune & Stratton Inc New York

ndelcorn M S Blankenship G & Machemer R (1976) Pars plana vitrectomy of evere diabetic retinopathy *Amer J Ophthal 81* 561–570

lalley C & Heintz R M (1975) Vitrectomy with an alternative instrument system *Ann Ophthal 7* 585–589

ebels R G (1978) Vitrectomy for complications of diabetic retinopathy *Arch Ophthal (Chicago) 96* 237–?46

yman G A Huamonte F U & Goldberg M F (1976) One hundred consecutive pars plana vitrectomies using the vitrophage *Amer J Ophthal 81* ?63–271

lentino F I Freeman H M McMeel J W & Schepens C L (1977) Closed vitrec

Material and Methods

The material comprised 92 patients ranging from 40 to 91 years of [illegible] operated for senile cataract. The assessment was based on a double [illegible] investigation. All patients had premedication consisting of 10 mg d[illegible] (Valium*) i.m. about one hour preoperatively.

The retrobulbar injection of 5 ml of the drug was made through the c[illegible] junctiva with a 3 cm long blunt needle.

Lidocaine 2 % with adrenaline (12.5 μg/ml) (epinephrine) was given to [illegible] patients, mean age 70 years. Etidocaine 1 % was given to 2[illegible] patients, mean a[illegible] 75 years. Etidocaine 1 % with adrenaline (5 μg/ml) (epinephrine) was give[illegible] 24 patients, mean age 72 years.

In all groups hyaluronidase (Penetrase*) was added to the anaesthetic a[illegible] at a concentration of 200 I.U./ml.

For facial nerve block 5 ml of the respective anaesthetic preparation w[illegible] adrenaline was injected in the pre-auricular area and along the facial ner[illegible] branch towards the orbicularis oculi. Various doctors performed the anaesthe[illegible] but the technique has been identical.

Average interval between the injection of the anaesthetic and the com[illegible] mencement of the operation was 18 min (12–20 min) in all the three grou[illegible] During this time the intraocular pressure was reduced by massage to 1[illegible] (Schiotz) or lower.

Table I
Effect of lidocaine and etidocaine
The need for additional injection in brackets

	Lidocaine 2 % adrenaline		Etidocaine 1 %		Etidocaine 1 adrenaline	
Satisfactory akinesia and anaesthesia	43		5		16	
Unsatisfactory akinesia of the eye lids	1	(1)	9	(3)	0	
Unsatisfactory akinesia of the eye bulb	1	(1)	0		5	([illegible]
Pain during operation	1		8		3	(1[illegible]
Total no. pat.	46	(2)	22	(3)	24	([illegible]

Results

..ble I comments concerning the effect of the anaesthesia and the need for ..l injection of the anaesthetic agents are summarized

..e table shows that unsatisfactory akinesia of the eye lids and the eye bulb ..unsatisfactory anaesthesia was observed more frequently in the two etido.. groups The difference is statistically significant ($P < 0.01$) Extra injec.. of the anaesthetic preparation was necessary in 2 of the 46 patients (4 4 %) ..e lidocaine group compared to 9 of the 46 patients in the etidocaine groups ..%) ($P = 0.02$)

..uring the postoperative phase pain relieving tablets were given to 14 ..nts (30 %) in the lidocaine group compared to only 6 patients (13 %) in ..tidocaine group The difference is on the border of statistical significance ..0.05) The need for pain relieving drugs occured on average 324 min after ..injection in the lidocaine group compared to 400 min in the etidocaine ..up

..here were no retrobulbar haemorrhages allergic reactions or local or ..ral toxic effects registered in any of the patients

Comments

..to the vasoconstrictory effect of adrenaline there have been misgivings ..cerning the use of this agent in an anaesthetic preparation to be used retro..barly mainly because of the danger of optic nerve ischemia (Thorburn et al ..6 Horven 19 8) Especially in the operative treatment of glaucoma with ..ere optic nerve damage one ought to consider omitting adrenaline in retro..bar anaesthesia The possibility of the general cardiovascular effect of ..enaline must be taken seriously in those patients with cardiac disease such ..coronary sclerosis and arythmia While some local anaesthetics including ..locaine contain adrenaline at a low concentration of μg/ml lidocaine ..contains 12 5 μg/ml

..With the retrobulbar anaesthesia and the facial nerve block and the interval ..ween the injection and operation used in this study lidocaine 2 % gave a ..er effect than etidocaine 1 % The necessity for analgesic drugs during the ..stoperative phase indicated a more protracted action of etidocaine than of ..ocaine These results may be explained by a more rapid onset with lidocaine ..in with etidocaine If this was the case the the difference in effect might ..ve been negligable if the interval between the injection and the operation ..d been of a longer duration However one of the main requirements of a

good local anaesthetic preparation is satisfactory anaesthesia and [illegible] which takes effect within the normal interval between the injection and [illegible] commencement of the operation

References

Castren J A (1963) A clinical evaluation of mepivacaine (Carbocain) in [illegible] surgery *Acta Ophthal Kbh 41* 26?–?69

Castren J A and Tammisto T (1966) A clinical evaluation of a new local [illegible] (Marcain – Adrenalin) in ocular surgery *Acta Ophthal Kbh 44* 83–[illegible]

Horven I (1978) Ophthalmic artery pressure Retrobulbar anesthesia *Acta O*[illegible] *Kbh 56* 574–586

Laaka V Nikki P & Tarkkanen A (1972) Comparison of bupivacaine with and [illegible] out adrenalin and mepivacaine with adrenalin in intraocular surgery *Acta Oph*[illegible] *Kbh 50* 229–238

Schlegel H J (1877) Lokale Langzeitanesthetika – eine Bereicherung fur die Op[illegible] mochirurgi *Klin Mbl Augenheilk 171* 359–370

Thorburn W Thorn Alquist A M & Edstrom H (1976) Etidocaine in Retro[illegible] Anaesthesia A comparison with Mepivacaine *Acta Ophthal Kbh 54* 591–6[illegible]

Author's address

Arne Ellingsson and Henry Aasved
University of Bergen Bergen Norway

Department of ophthalmology (Head G Scuderi)
Radiology Section University of Rome Italy

IMPROVED RADIOLOGICAL TECHNIQUE FOR EVALUATING THE LACRIMAL PATHWAYS WITH SPECIAL EMPHASIS ON FUNCTIONAL DISORDERS

BY

ARRIGO MONTANARA, PIETRO CATALINO and MASSIMO GUALDI

A radiological study of the lacrimal drainage apparatus was made in 50 patients with temporary or permanent epiphora The authors state that the present radiologic technique (macrography seriography subtraction) allows the detection on the various lacrimal levels of not only organic lesions but also of functional disorders They assert the definite superiority of dacryocystography over the other clinical and radiological investigations (scintigraphy roentgencinematography) in identifying the site and the nature of the various organic lesions in evaluating and interpreting the functional alterations as well as following and judging the effectiveness of treatments

Key words Lacrimal drainage apparatus – Radiological features of tear flow disorders – Macrodacryocystography – Lacrimal Passages

Abnormalities of lacrimal function have been given different names by the various authors functional blocks (Demorest & Milder 1955) physiological dysfunctions (Hurwitz & Welham 1975) partial functional obstructions (Montanara et al 1979a) functional disorders (Montanara et al 1979b) All of these conditions share the characteristic that the anatomic patency of the lacrimal passages is not associated with functional patency To our knowledge this subject has not been systematically discussed from a radiologic viewpoint and

Received August 8 1978

no adequate review has been made of its various aspects and of the d
material obtainable in such cases The purpose of this paper is to pre
review and discussion of the subject

Clinical Tests Lavage and incannulation the various taste (saline,
and colour tests (Lipsius 1957 Vergez 1961 Jones 1967 Norn 19 Le
Jones 1968 Murube del Castillo 1973 Hurwitz & Welham 1975) (princ
the Jones fluorescein dye tests) lacrimal electromanometry (Callahan e
1965) while under certain circumstances prove to be of considerabl d
gnostic value do yield results that are not always consistent or in agre
with each other Such tests may simply demonstrate an anatomic patency
do not allow definite judgement of the functional integrity of the s
(Zappia & Milder 1972) Moreover these methods do not demonstrate
absence (Hurwitz & Welham 1975) or pinpoint the precise location of
abnormality

Radiological investigations Lacrimal microscintigraphy (Rossomondo
1972 Carlton et al 1973 Chauduri et al 1974 Sorensen & Taagehøj
and quantitative scintingraphy (Hurwitz et al 1975) allow an excellent
ation of lacrimal function and from this viewpoint are superior to the
clinical and radiological methods for evaluating the dynamic of tear flow
for assessing lacrimal excretory function In the clinical field however
evidence thus obtained does not give reliable information concerning the
of the condition studied nor a determination of the precise location
stenosis and surgical indications are therefore uncertain These tests a
superb for physiology are still not as precise as dacryocystography in de
anatomical abnormalities Scintigraphy is therefore to be regarded
opinion as complementary to macrodacryocystography which in a
should be performed for the purposes of diagnostic confirmation.

The interest of dacryocinematography (Street & Howell 19 Trol
Potter 1970) is limited mainly to the area of scientific research since
help to elucidate the phases of excretion through an analysis of the
mechanisms acting during tear drainage that are still under discussion (
lary attraction blinking of the eyelids gravity force nasal aspiration) F
more roentgencinematography also requires for diagnostic confirmat
follow - up dacryocystography since it provides very limited morph
information due to the fact that the canaliculi are not clearly visible b
of their small size Also the images of the sac and of the lacrimo nasal d
pear to be lacking in detail due to factors that are inherent (Montanara 1
the method itself



Fig 1

acrimal seriography show a constriction of the internal tract of the right hand com on canaliculus due to the presence of a mucous membrane (arrows) the filling defect always present in the various seriograms The passage of the contrast medium into e lacrimal sac is unimpeded the sac appears moderately ectasic due to inflammation nother functional constriction exists in the lower tract of the sac (arrow) only an erall analysis of the various seriograms provides a visual impression of the difficulty of the distension of the lacrimal walls which retards the drainage

To the left obstruction of the sac

Dacryocystography (DCG or serial dacryocystography or macrodacryocystography are synonymous) This technique in our opinion, allows the complete evaluation of lacrimal drainage In the *canaliculi* exclusive functional changes are uncommon whereas these are more frequent beyond the internal opening of the common canaliculus Epiphora and functional disorders under such circumstances may be due to a small mucous membrane (Welham & Henderson 1973) located in the common canaliculus, in two thirds of the case (Rycroft 1960) in the inner part (Fig 1) usually because of inflammation of the sac The precise location of the different alterations can be left undetermined if the examination is conducted without subtraction (Keast Butler et al 1973)

In the *sac and lacrimo nasal duct* the delayed drainage is most frequently of functional origin In this area the promoting factors are the malformations

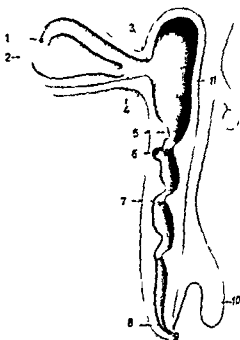


Fig 9

Distribution of the various valve like formations (actually thickenings of the mucosa considered) described in the lacrimal passages 1) Bochdalek s valve 2) Foltz valve 3) Rosenmuller s valve 4) Mayer s sinus 5) Beraud Krause s valve 6) Arlt s sinus 7) Taillefer s valve 8) Hasner Cruveilhier s valve 9) lower orifice of the lacrimo nasal duct 10) lower nasal horn 11) insertion of the palpebral ligament

imple variants of the bony structures (unguis ethmoidal cells nasal fossa) more frequently the drainage defects are due to an inflammatory process he lacrimal or nasal mucosa or to a simple mucous hypertrophy perhaps allergic origin It should be mentioned here that the mucous membrane called valves) which lines the sac and the duct causes dilatation and con tions variously described by the authors after whom the different valve formations are named (Fig 2) These should actually be regarded as simple kenings of the mucosa since neither anatomic preparation nor radiological estigations have confirmed the presence of any such valves

Material and Methods

nks to progress in radiological technique (Campbell 1964 Iba & Hanafee 1968 es 1974 Lloyd 1975 Montanara et al 1979) it is not possible to detect the presence only of organic lesions but also of functional disorders of the lacrimal drainage em. To demonstrate such alterations the investigations of choice should be *dacry io aphy* performed under the following conditions

Bilateral introduction of the contrast medium while the films are obtained
Seriography
Direct enlargement (focal spot of small size not more than 0 3 mm)
Ima e subtraction

The use of a catheter or needle connected to a catheter protects the operator by owing him to keep at an adequate distance from the radiation source the dose eived by patient is relatively low with the technique used (80 kV 15 mAs)

We have no information in literature that any dosimetric researches on the amount radiation received by the cornea and the lens during the DCG have been per rmed. With the technique we use the bulb is affected by a comparatively low nount of radiation certainly not superior to that absorbed during a conventional ray examination of the skull This in fact needs an almost identical number of diograms and a higher value of mAs in comparison with the DCG with this last chnique we make a direct magnification and do not use the ratiographic grid In is way the DCG does not provoke a particular risk for the patient The problem the dose maybe is of concern to the radiologist particularly if he is not equipped oth with an automatic skull seriography and an automatic injector This is why we dvise both local lead protections applied on the skull table and use of a catheter hich connected to the lacrimal needle allows the operator to stand comparatively ar enough away from the x ray tube

Fifty patients with lacrimal problems (temporary or permanent epiphora) have been adiologically studied by means of macrodacryocystography In 40 subjects the hysical exam nation syringing of the lacrimal passages and the taste and colour ests were negative ten patients presented clinical findings of unilateral stenosis

The contrast medium used was the ultrafluid Lipiodol advisable because it is homogenous non irritant non toxic is eventually absorbed if not discharged and does not produce a bitter taste Also it will not form a powdery deposit on the lids

with subsequent burning as may happen when using water soluble contra[illegible] The ultrafluid Lipiodol s viscosity (?5 centiposes at 37 C) is higher than tears [illegible] 2 916 centiposes Hurwitz & Welham 1975) The water solubl[illegible] lower viscosity Conray 4 centiposes at 37°C Angiografin in 6[illegible] solution [illegible] poises at 37°C which is within the normal range for tears Using the water [illegible] solution diluted by tears it was not easy to demonstrate radiolo[illegible]lly the [illegible] material in the pharynx thus we prefer to use ultrafluid Lipiodol in functional [illegible] of the kind There were no adverse reactions to this contrast medium

All examinations were performed in the antero posterior position The patient [illegible] placed supine on the skull table and some drops of local anaesthetic are instilled [illegible] the conjunctival sac Lloyd s technique is based on a 1° inch intravenous [illegible] bilaterally inserted into the canaliculus we prefer to place a lacrimal needle [illegible] canaliculus (lower or upper) and connect it with the catheter Two or three [illegible] ultrafluid Lipiodol are injected in each lacrimal ductus In order to obtain op[illegible] demonstration of the duct system it is essential not to advance the tip needle [illegible] than 3 to 4 mm into the canaliculus (Fig 3) It is the usual practice to carry [illegible] bilateral introduction simultaneously in this way useful information can be [illegible] by comparison with the opposite side

Immediately following the scout film (performed prior to the injecti n for sub[illegible] tion purposes) two sequential radiograms are taken during the ints [illegible] of the contrast medium In patients with lacrimal physiological dysfunctions we found [illegible] films taken 30 sec and 1 3 and 5 min after instilling Lipiodol gave the [illegible] information

Geometrical enlargement of the radiographic image is obtained by placin[illegible] the [illegible] drawn serial changer some distance from the patients head With co ope[illegible] and a head fixation device substraction studies may easily be achieved

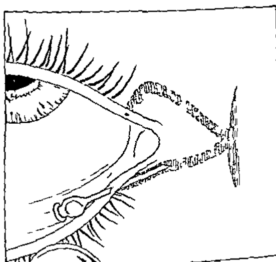


Fig 3
Correct position of the needle into the canaliculus

Results

ne 50 patients (100 lacrimal systems examined) we classed 10 of these as ologically normal Another 10 subjects presented a complete or incomplete iteral stenosis located at the level of the common canaliculus (2 cases) or nd the internal opening of the common canaliculus (8 cases)

ne lacrimal systems with purely functional abnormalities and with indica radiological features were therefore 70 The final results are shown in le I

o evaluate Lipiodol s drainage time it is evidently important to know the time in normal subjects In researches performed by one of us (Mon ra 1979) concerning 80 lacrimal systems (40 subjects who showed no omical or functional lacrimal alterations and were tudied both with ultra d Lipiodol and water soluble contrast media) we found that the drainage he lacrimal system begins a few seconds after Lipiodol s introduction and emptyng into the nasal fossa occurs within 6–15 sec (10 sec as a mean) as wn in Table II

n the normal subjects studied with water soluble contrast media (Conray

Table I

Radiological features		Lacrimal systems examined
Normal lacrimal drainage apparatuses		20
Anatomical alterations (unilateral stenosis or ob truction) The opposite side presented constantly functional disorders		10
Functional disorders	a) sac deformed or deviated (unilateral)	11
	b) disproportion between the relative diameters (unibilateral)	12
	c) alternating dilated and constricted tracts (unibilateral)	15
	d) skipping of a tract (unilateral)	7
	e) prolonged drainage time (unibilateral)	25
Total		100

Table II
Flow time of the contrast media in normal subjects

Normal lacrimal systems examined	Angiografin Conray 35 %	ultrafluid lipiod l
80	40	40
initial drainage time (minimum maximum)	Mn = 2 seconds Mx = 4 seconds	Mn 6 seconds Mx 15 seconds
mean drainage time	2 2 seconds	10 seconds

or Angiografin diluted at 35–40 %) the drainage time was found to be e shorter two sec after introduction the contrast medium was already presen the nasal fossa (Table II and Fig 4)

The persistence of the contrast medium in the sac and in the duct completely opacified after more than 30 sec from the introduction wit any passage into the nasal fossa in our opinion should be regarded as of functional disorders It is not possible to standardize the sequence of the various radiograms as a general rule we recommend carrying out a serogr (2 films in addition to direct examination) in the first 10 sec during the

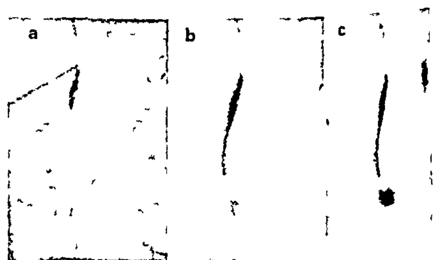


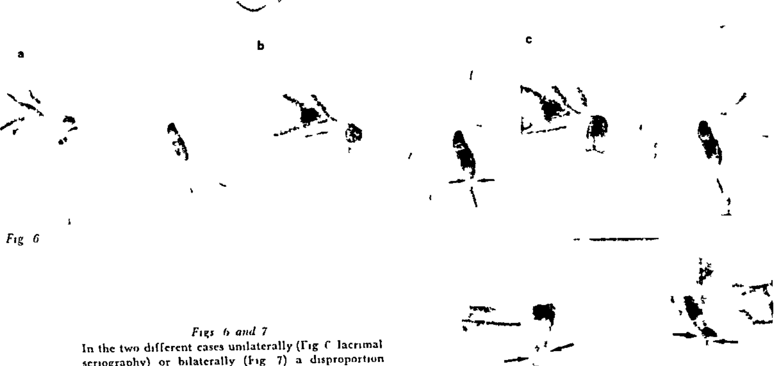
Fig 4
A normal case studied by automatic seriography (two images/sec) and subtraction At the end of the 2nd sec the contrast medium (water soluble) opacized the nasal fossa

f the ultrafluid Lipiodol and subsequent control films at 1–3–5 min The of 30 sec which was chosen as a parameter to determine the discharge as logical represents the minimal value required to suspect the presence of onal disorders More reliable elements of judgement are offered by ssive seriographical controls eventually performed after 1–3–5 min fol the introduction of the contrast medium



Fig 5

acrimal seriography On the left side the sac appears off axis (arrow) and slightly igled outwards (see comparison with the healthy side) The contrast medium stops the level of the lower segment of the sac and later of the lower tract of the duct



Figs 6 and 7

In the two different cases unilaterally (Fig 6 lacrimal seriography) or bilaterally (Fig 7) a disproportion (→ ←) is noticeable bet veen the diameter of the sac and that of the du t below it (in Fig 6 on the right sid a lacryo ystitis combined with a ompl te l tr t on of the ac) In both a the lra nage t l l (l n n)



Fig 8

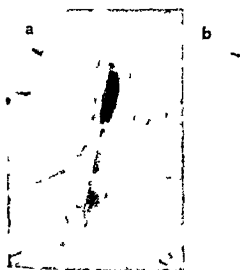


Fig 9

Figs 8 and 9
the two different cases an overall distended appearance of the right hand lacrimal passages is noticeable with delayed drainage into the nasal fossa (over 2 min)

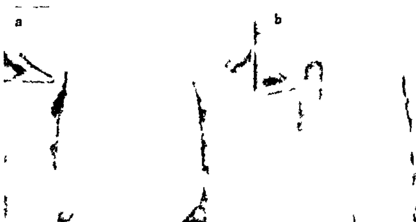


Fig 10 A B

The lacrimal system show in both different cases dilated tracts alternating with constricted ones Delayed drainage (5 min) In Fig 10 B on the right side there is complete obstruction of the sac

a b c

Fig 11

The sac appears moderately dilated and the drainage is retarded (9 min) A slip is noticeable in the filling of the lacrimal lumen and the point of passage between sac and duct never shows up in the various seriograms (arrow)

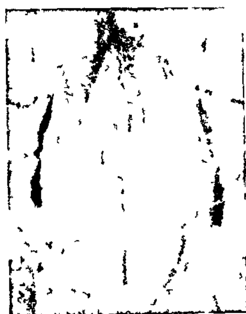


Fig 12

drainage of the lacrimal passages is slowed (over three min) by stoppage of the contrast medium on the level of the lower tract of the lacrimo nasal duct

i the cases of functional disorders the sac does not appear to be particu / dilated on film The radiological features are different in the various s and may be summarized as follows

The sac appears to be of normal size but deformed and or barely deviated n the axis or sometimes also angled the asymmetry can be easier detected omparison with the healthy side (Fig 5)

The sac shows a moderate dilatation which by itself may not be of hological significance and could go undetected by carefully examining the meter of the lacrimal segments however it is found that a disproportion sts between the diameter of the sac and that of the downstream duct (Figs) without the picture changing in the various seriograms

The sac and the duct appear more or less dilated partly or completely netimes presenting a frozen appearance (Figs 8–9) in other cases con icted sections alternate with wider ones (Fig 10)

The contrast medium skips a given tract (Fig 11) which therefore never comes opacified

5) The sac and the lacrimo nasal duct are of regular sh pes and ... dramage time is more or less prolonged (always longer tha 3) ... proximately in one third of our cases this fact constitutes the only p... element and the contrast medium stops for such a time at a given ... level (Fig 12) or at several levels

Discussion

Of the 100 lacrimal systems examined concerning patients affected by e... phora seventy presented probabely purely functional disorders. In ... can reasonably exclude anatomical lesions such suspicion did not exist ... clinicals tests and the DCG showed neither dilatation nor remarkabl de... formations of the lacrimal pathways On the other hand the existence of ... co existence of the morphological signs illustrated on Table I and the ... tion of the delayed drainage imply that the epiphora is d e to disease of ... functional nature Naturally the morphological signs which have been de... scribed have a relative value as with all other signs in radiology ... detection of them can be useful because focusses attention on a func... pathology The extended drainage time is of great importance for the ... gnostic effects and yet this can be considered pathological only if w ... the exact normal time discharge of that particular contrast medium (Tab ...

Now let us consider the causes responsible for the functional disorders ... may suppose there is some relation of functional disorders and ... alterations to inflammation at least a chronological one. The original ... may even be due to a slight inflammation of the lacrimal mucosa which ... not provoke ectasias or deformations and is thus hardly remarkable on ... examination especially when this is performed according to the conve... technique With time the inflammation stresses the functional disorders ... these in turn enhance the inflammation Afterwards through a mechan... similar to those occurring in other apparatuses (i e in the digesture tract ... functional diseases may be transformed into organic ones inflammation ... functional alterations further increase causing anatomical damage which c... either be reversible or not

At the DCG exspecially at the level of canaliculi the inflammatory f... are difficult to define Furthermore as for as the drainage time is concer... we have few methods of evaluating this unless the seriographic techniq... adapted On the contrary by means of the serial DCG with subtraction we ... advance the diagnosis in the first period of early inflammation it is po...

ake the diagnosis in the second period of the functional disorders it is to make the diagnosis with the DCG then in the third period of anatomi lterations the diagnosis is *extremely easy* to make and can also be made onventional dacryocystography

Conclusions

frequent reactions of the ophthalmologist – particularly if the canaliculi involved – is characterized either by a discouraged lack of interest or by scepticism about the change of curing the epiphora This attitude which itably affects the patient leads to inactivity or abstention from treatment finds its main support in the fact that the symptoms are neither severe *painful though* complications may become so The *initial difficulties* con ed with the epiphora are of an aesthetic nature and more or less well rated depending on the disposition of the patient But since these cases r good therapeutic possibilities this fatalism does not seem to be justified either physician or by patient

he treatment for the alterations sited in the canaliculi is largely surgical ile therapy of functional disorders in the sac and duct is often medical er proper diagnostic recognition the causes responsible for the mucous ertrophy and the epiphora can be approached more effectively Occasional however these conditions are more the domain of a medical clinician than t a specialist (ENT Ophthalmologist) since abnormalities of lacrimal pas es can sometimes be part of localisation of allergic or toxic disorders ecting the entire body

A precise knowledge of the radiological features present in such disorders – and with increasing frequency as our experience expands – allows a better derstanding of many cases of epiphora previously unrecognized since the rious diagnostic clinical and radiological examinations may be negative It desirable therefore that such functional disorders be recognized and better derstood by both radiologists and clinicians alike The former should arrive the correct diagnosis using all investigative techniques and methods called r by the individual case while the clinicians should decide on the choice of eatment more likely to be therapeutically effective

References

Callahan W P Forbath P G & Besser W D S (1965) A method of determining the patency of the naso lacrimal apparatus *Amer J Ophthal 60* 475–481
Campbell W (1964) The Radiology of the lacrimal system *Brit J Radiol* 37 1–
Carlton W H Trueblood J H & Rossmondo R M (1973) Clinical evaluation of microscyntigraphy of the lacrimal drainage apparatus *J Nucl Med 14*
Chaudhuri T K Saparoff G R Dolan K D & Chaudhuri T K (19) A comparative study of contrast dacryocystogram and nuclear dacryocystogram *J Nucl M* 16 605–608
Ciabattoni P & Montanara A (1978) L indagine radiologica delle vie lacrimali studio dei canalicoli *Radiol Med 64* 961–976
Demorest B H & Milder B (1955) Dacryocystography The pathologic lacrimal apparatus *Arch Ophthal (Chicago) 54* 410–421
Hurwitz J J & Welham R A N (1975) Radiography in functional lacrimal testing *Brit J Ophthal 59* 323–331
Hurwitz J J Massey M N & Welham R A N (1975) Quantitative lacrimal scintillography I Method and physiological application II Lacrimal pathology *Brit J Ophthal 59* 308–322
Iba G B Hanafee W N (1968) Distension dacryocystography *Radiology* 90 1020
Jones B Personal communication 1959 Quoted by Lloyd G A S Welham R A N (1974) Subtraction macrodacryocystography *Brit J Radiol* 47 379–382
Jones L T (1962) The cure of epiphora due to canalicular disorders. Trauma and surgical failures on the lacrimal passages *Trans amer Acad Ophthal Otolar* 66 506–512
Keast Butler J Lloyd G A S & Welham R A N (1973) Analysis of ... macrodacryocystography with surgical correlations *Trans Ophthal Soc U K* 593–596
Linn M L & Jones L T (1968) Rate of lacrimal excretion of ophthalmic ... *Amer J Ophthalm 65* 76–80
Lipsius E I (1951) Use of sodium saccharin for testing the functioning of the lacrimal passages *Amer J Ophthal 43* 114–115
Lloyd G A S (1975) *Radiology in the orbit* Saunders W B London
Montanara A (19 6) *Elementi di radiodiagnostica oftalmologica* Ed Minerva Medica Torino Italy
Montanara Modugno G C & Ciabattoni P (1979a) Importancia de la exploracion radiologica en las alteraciones organicas y funcionales de las vias lagrimales *Soc Canaria de Oftalmologia* (in press)
Montanara A Ciabattoni P & Rizzo P (1979b) Stenoses and functional disorders of the lacrimal drainage apparatus *Survey of Ophthalmology* 23 249–258
Murube del Castillo J (1973) Test for spontaneous canalicular functioning *Arch Ophthal 1* 61–66
Norn M S (1966) Tear secretion in diseased eyes *Acta Ophthal (Kbh)* 44
Rossomondo R Carlton W H Trueblood J H & Thomas R P (19) A new method of evaluating lacrimal drainage *Arch Ophthal* 88 523–525
Rycroft B W (1960) Observations on corneoplastic surgery *Proc roy Soc Med* 303–310

..en T & Taageboj F J (1977) Methodological aspects of tear flow determina
ı by means of a radioactive tracer *Acta Ophthal (Kbh)* 55 726–738
t D F & Howell M H (1967) An alternative radiographic technique for
crodacryocystography *Brit J Radiol 40* 235–238
el S L & Potter G D (1970) Kinetic dacryocystography *Amer J Ophthal 70*
1010–1011
ez A (1961) Contribution au diagnostic et au traitement non operatoire des af
tions des voies lacrymales *Ann oculist 194* 193–216
ıam R A N & Henderson P H (1973) Results of dacryocystorhinostomy analysis
causes of failure *Trans ophthal Soc U K 93* 601–609
ıa R & Milder B (1972) Lacrimal drainage function *Amer J Ophthal 74* 154
9 160–167

ıor s address
Arrigo Montanara
Gorizia 9?
ıa Italy

Department of Ophthalmology (Head N Ehlers)
and Department of Nuclear Medicine Isotope Unit Radiumcen.
(Head H Hvid Hansen) Århus Kommunehospital
University of Aarhus Denmark

TEAR FLOW IN NORMAL HUMAN EYES DETERMINATION BY MEANS OF RADIOISOTOPE AND GAMMA CAMERA

BY

T SØRENSEN and F TAAGEHØJ JENSEN

Tear flow was measured in normal human eyes by means of a radioisotope (technetium as pertechnetate in a normal saline solution) a gamma camera and a computer By region of interest technique the elimination was shown to have two phases an initial rapid elimination followed by a slower elimination after 5–7 min The mean fractional turnover rate in the initial phase was 0 197 min $^{-1}$ (n = 35 SEM = 0 018) and in the basal phase 0 053 min $^{-1}$ (n = 35 SEM = 0 003) Assuming a constant tear volume these values corresponded to a tear flow of 1 4 μl min $^{-1}$ and 0 6 μl min $^{-1}$ respectively There was no significant difference between the fractional turnover rate in the upright and supine position No difference was found in tear flow between males and females With the eyes closed the fractional turnover rate was low with intermittently rapid outflow of tears Irritation to the contralateral eye with a filterpaper caused a stimulated tear flow of 4 4 μl min $^{-1}$ A nomogram facilitating background corrections was constructed

Key words Tear flow – human – technetium – gamma camera background correction

Measurements of tear secretion have been carried out by several authors w various results Values from 0 2 to 100 μl/min. have been reckoned The wi varying results are not surprising considering the conditions under which tears were collected In methods using a filterpaper a stimulated tear secr is measured The amount of tears in the filterpaper is however highly depe

Received December 11 1978

n the outflow of tears through the tear drainage system and the amount is outflow is totally unknown A stimulation to the eye will cause a rapid flow only for a few minutes (Mishima et al 1966 Sorensen & Taagehoj n 1975) The varying results consequently depend on the time of measure i e the longer the determination time the smaller the influence of the il high tear flow In Table I are outlined the results of some previous es Schirmer (1903) is usually quoted having found a tear secretion of 0 5– gr/16 h In fact he found 0 2–0 4 gr/16 h and an evaporation of 0 2/ gr/ When comparing Schirmer s results with the values in Table I the eva tion should not be included According to later studies on rabbits Mishima aurice 1961 and Iwata et al (1969) carried out under more physiological umstances Schirmer s evaporation rate was almost three times too high

n excellent study on the dynamics of tear flow was presented by Mishima l (1966) using fluorescein and a fluorphotometer As a new finding they ved that the elimination of fluorescein fairly well fitted to the expotential ern of elimination which had been assumed in other studies (Kirchner d Brandt & Fritsche 1967) Mishima et al also were first to demonstrate a iasic elimination with an initial high flow an a lower flow after 4 to 5 utes

n 197? Rossomondo et al introduced lacrimal scintigraphy by means of a oisotope and a gamma camera Quantitative data on the dynamics of tears

Table I

ear flow found by various authors The values are not corrected for evaporation

Author	Method	Tear flow (μl/min [1])	Determination time
irmer (1903)	filter paper	0 ?–0 4	0 ı5–2.5 hours
lik (195?)	filter paper	15	5 min
ver & Jaeger (195?)	dye dilution	10	2 min
chner (19?4)	dye dilution	0 3–0 9	0 5 hours
rn (1965)	dye dilution	10–20	5 min
ish ma et al (196?)	dye dilution	1 2	7–15 min after instillation
andt & Fritsche (196?)	dye dilution	6 6	3 min
llers (196)	calculated	2 4	
rensen & Taa ehoj Jensen (present study)	Tc dilution	0 6 (0 5 see text)	7 5–15 min. after instillation

Table II
Tear flow studies using Tc[illegible] as tracer

Author	Vehicle	No of determinations/persons	Volume	Radioactivity	Evaluation
Dressler & Denffer (1974)	Tc pertechnetate	not revealed	20 µl	[illegible]0 µCi	half time 1 min (in conjunctival sac)
Dressler & Denffer (1975)	Tc sulphur colloid	10 eyes	10 µl	50 µCi	half time 1 min (in conjunctival sac)
Carlton (1978)	Tc (vehicle) not revealed	28 persons	15 µ	100 µCi	half time 56 sec (in conjunctival sac) median transit time to the bottom of the nasolacrimal duct 48 sec (4–323)
Hardberger et al (1975)	Tc pertechnetate in saline	4 eyes	[illegible] µl	not revealed	half time 4 6 min (region of interest not defined)
Hurwitz et al (1975)	Tc sulphur colloid	7 persons	13 µl	130 µCi	erect half time 4 1 min (n = 7) supine half time 38 0 min (n = [illegible]) (region of interest was inner canthal area)
M[illegible]yer & Dau[illegible]ch (19[illegible])	Tc perte[illegible]hn[illegible]tate	73 per[illegible]n	2 and 10 µl	[illegible] µCi	[illegible]

a similar equipment have been published by several authors (Table II) e results are not directly comparable owing to differences in regions of est evaluation methods vehicles and instillated volumes Due to the l elimination during first minutes after instillation the reckoned half times ot be converted to a fractional turnover rate However the tendency is the radioisotope introduced into the conjunctival sac very rapidly leaves a the tear pathways

is paper describes the results of dynamic tear flow studies in normal an eyes using a radioisotope and a gamma camera with computer assist the results being expressed as fractional turnover rates

Material

volunteers were selected from the hospital staff students and myopic ents who were later fitted with soft contact lenses In 35 normal persons males 20 females) a determination was carried out in one eye only A nd determination was performed in six persons with a few days interval with only five min interval in 13 persons

Methods

another paper (Sørensen & Taagehoj Jensen 1977) the method has been described detail

he patient was placed in a supine position with his eye approximately 3 cm from the hole collimator of the gamma camera i e 30 cm from the scintillating crystal µl of a normal saline solution containing approximately 900 µCi technetium m) as pertechnetate was placed on the center of the cornea without local anaesthe A scintigram was taken at the beginning and at the end of 15 min measuring time unveil a possible displacement of the eye or trapping of the radioisotope in the lashes

By serially radioactivity recordings at ten sec intervals from the designated con nctival sac area (region of interest technique) an elimination curve was con ucted by the computer and plotted on a semilogarithmic system From the approxi ated curves in the initial and basal phases the fractional turnover rates were calculat (Sørensen & Taagehoj Jensen 1977) (See Fig 3)

In the investigations on subjects in the supine and upright position a newer equip ent not principally different from the detection system used in the other deter inations was utilized (new detection system gamma camera Ohio nuclear ON–100 ahole collimator interfaced minicomputer Varian 620/L–100) With this new detec on system background corrections were not necessary

In 93 persons a Schirmer filter paper reading was taken to correlate this estimation f tear secretion to the fractional turnover rate The filterpaper standardised by Hal

berg & Berens (1961) was placed in the lateral third of the lower lid and e.h. ... after five min or in patients with high lacrimation read as the total wetting of ... filterpaper (30 mm) per time required for the total wetting

The comparative tear flow determinations in the supine and upright position w... separated by an interval of several days

The elimination of technetium was followed in nine normal persons placed in t... supine position with closed eyes

In three persons the stimulated fractional turnover rates were determined by pl... a filter paper strip in the fellow eye to the one being investigated

Background correction

The contribution from the background components has been evaluated in an ... paper (Sørensen & Taagehoj Jensen 197/) In this study corrections were calc... for the natural background B_n and the background B_t due to the absorption thr... the conjunctiva and recirculation to the region of interest (B_t is the activity at t... t and B_f the final value of B_t at time ∞)

The fractional turnover rate from the first determinations were corrected accor... to equation (1)

$$Q_t = Q_o\, e^{-k\,t} = A_o\, e^{-k_1\,t} + B_n + B_f\,(1-e^{-0.015\,t})$$

When double determinations with five minutes interval were done the second fr... tional turnover rate was corrected according to equation (?)

$$Q_t = Q_o\, e^{-kt} = A_o\, e^{-k_1\,t} + B_n + B_f\,(1-e^{-0.015(t+?0)}) + B_f\,(1-e^{-0.015\,t})$$

Q_t is the recorded activity at time t
A_o the activity representing the deposit at time 0
k the estimated fractional turnover rate on condition of no background components
k_1 the fractional turnover rate corrected for background components,
t the time in minutes
e the base of the natural logarithm and
0 015 is the fractional turnover rate found in absorption studies on normal h... persons (Sørensen & Taagehoj Jensen 1979)

The corrections for these background components is shown graphically in Fig 1

Results

As shown in Fig 3 an initial very rapid elimination was found in the first ... min following instillation due to the outflow of the surplus volume of t... Then a steady state was present for a few min seen as a straight line o... semilogarithmic plot (= initial phase in Table III) The following part of ... elimination from 7 to 15 min was called the basal phase Because a st... state cannot be assumed in the first 1–2 min a tear flow has not been calcul... for this phase

The fractional turnover rates in the initial and basal phases are shown ... Table III and Figs 1 & 2 The mean basal fractional turnover rate was f...

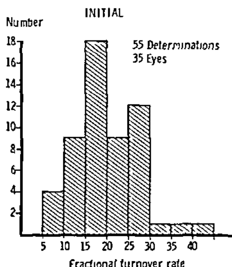


Fig 1

ctional turnover rates in the initial phase in normal human eyes Dimension on abscissa •• min⁻¹

Table III

Normal values for fractional turnover rate

phase (closed eyes)	
onal turnover rate (one eye in 35 persons)	0 083 min⁻¹ (SEM 0 0034)
onal turnover rate (one eye in 19 persons)	1 determination 0 085 min ¹ (EM 0 0049)
	2 determination 0 0 4 min ¹ (EM 0 00 0)
	(t test for paired data °P = 5–10 •
phase (open eyes)	
onal turnover rate (one eye in 35 persons)	0 197 min⁻¹ (SEM 0 013)
onal turnover rate (one eye in 20 persons)	1 determination 0 208 min ¹ (SEM 0 019)
	? determination 0 178 min⁻¹ (SEM 0 014)
	(t test for paired data _P = 1–⁰⁶ •
phase (open eyes)	
ional turnover rate (one eye in 9 persons)	0 048 min⁻¹ (SEM 0 011)
elimination phase (closed eyes)	
ional turnover rate (one eye in 5 persons)	0 159 min ¹ (SEM 0 0 0)

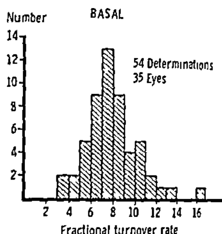


Fig 9

Fractional turnover rates in the basal phases in normal human eyes Dimension abscissa %/ min−1

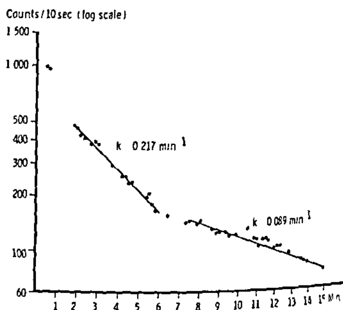


Fig 3

Activity time curve from the conjunctival sac area Dots represent radioactivity in ten second interval Solid lines are appromated curves The fractional turn over rates in the initial and basal phases are indicated with a k (In this example the background correction was high see Fig 10)

0 083 min⁻¹ With the assumption of a tear volume of 7 μl in the con ...nal sac (Mishima et al 1966) the tear flow could be calculated to be ...l min ¹ neglecting the absorption to the blood and the evaporation of ...r The fractional turnover rate in the initial phase was found to be ... l min ¹ corresponding to a tear flow of 1 4 μl min⁻¹

...diphasic elimination in a semilogarithmic plot was found in all 54 deter...itions with the exception of 3 that turned out to have a monophasic eli...ition There was no obvious explanation for this monophasic elimination ...rn except in one patient who suffered watery eyes outdoors

...i double determinations the second determination had smaller fractional ...r rates especially in the initial phases (t test for paired data 2p = 1–2% ...0) for determinations with days and 5 min intervals The difference was ...ificant in double determinations with 5 min intervals (t test for paired data 0.5–1 0 %, n = 13) and not significant with days interval between the meas...nents In the basal phases this tendency was less pronounced (t test for paired ...i ? p = 5–10 %, n = 19) for determinations with days and 5 min intervals and = ?.5–5 0 % (n = 13) for 5 min interval determinations)

...o difference was found between males and females neither in the initial ...in the basal phase The range of age was 15–40 years (most of the persons ...e 20–30 years of age) and therefore the age flow correlation was not ...estigated

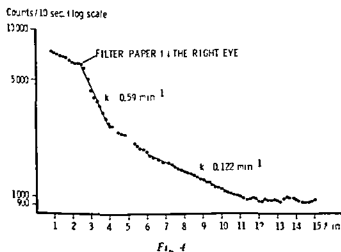


Fig. 4

...ctivity time curve from a normal left eye during stimulation to the right eye by a filterpaper at the time marked by the arrow

Table IV

Fractional turnover rates in the supine and errect position in the same pe[illegible]

Fractional turnover rate (mean ± SEM)

	Supine	Erect
Basal (n = 7)	0 079 ± 0 008 (min $^{-1}$)	0 063 ± 0 0[illegible] (min [illegible]
Initial (n = 7)	0 164 ± 0 013 (min $^{-1}$)	0 14[illegible] ± 0 0[illegible] (min [illegible]

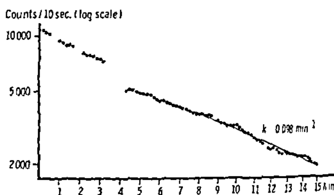


Fig 5A

Activity time curve from an eye not slightly turned to the side opposite to [illegible] being investigated Note the small fractional turnover rate and the less pr[illegible] rapid outflow in the first seconds after installation.

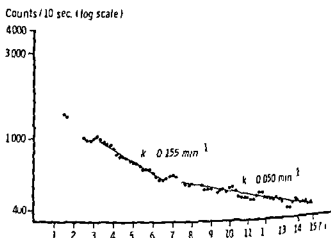


Fig 5B

From the same person as in Fig 5A the head being turned correctly

s method caused only a small stimulation to the eye A more stimulated low was demonstrated by performing a Schirmer filterpaper test in the eye to the one being investigated In Fig 4 is the moment of the insertion filterpaper marked by the arrow The stimulated tear flow was 4 4 μl [1] (mean fractional turnover rate 0 63 min^{-1} n = 3)

e fractional turnover rates were determined in seven persons in the supine upright position in the same eye The results are outlined in Table IV was no significant difference between the fractional turnover rates er in the basal nor the initial phase 2p 0 1–0 2 and 0 4–0 5 respectively) described in an earlier paper (Sorensen & Taagehoj Jensen 1977) the s of the volunteers were sligthly turned to the opposite side of the eye investigated to avoid pooling of the radioisotope in the outer canthal If the head was placed with the nose perpendicular to the ceiling or tly turned to the side of the investigated eye an elimination curve with a rent shape was seen as illustrated in Figs 5A & 5 B The rapid outflow in irst 1-2 min is not present and the curve is not diphasic

is head tilt in the supine position was of paramount importance With the

Fig 6 4

Scintigram of an eye without turn ng the head to one side

Fig 6B
Scintigram of the same eye with the head correctly slightly turned to the side opp
to the investigated eye

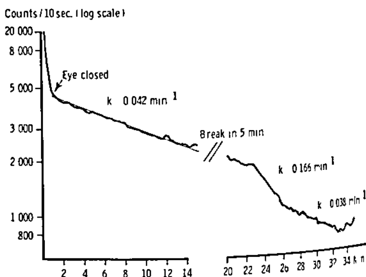


Fig 7
Activity time curve from a person with closed eyes The person blinked frequen ly d
first minute Then the eyes were closed for 34 min A rapid elimination phase
is seen from 23 to 26 min

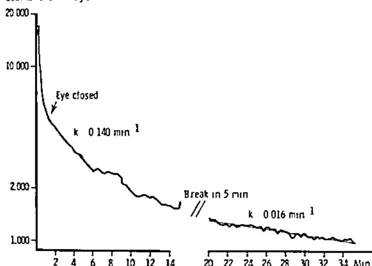


Fig 8

ed eye activity time curve from another person (see text in Fig 7) Rapid eli ition phase is less pronounced (9 to 10 min) Diphasic shape as in normal open eyes is seen with the change at 6 min after instillation.

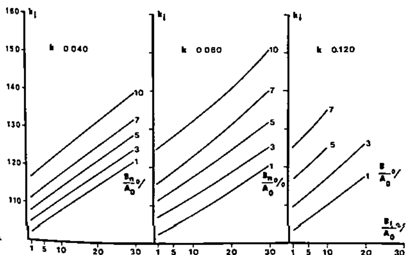


Fig 9

omogram for background corrections Ordinate Corrected fractional turnover rate in rcent of non corrected value Interpolation can be done for values not referred See text for further explanation

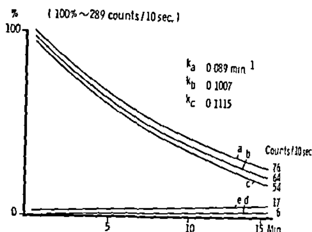


Fig 10

Graphical illustration of background correction (values from Fig 3) Curve a ... curve approximated to the actual curve in the basal phase Curve c and d are the back ground curves according to equation (1) and (2) Curve b = a − d and curve c ... the first and second determination respectively

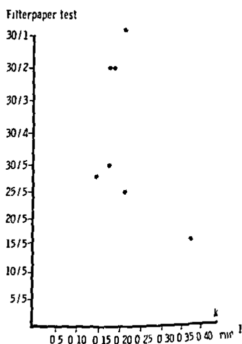


Fig 11

Correlation between filterpaper test and fractional turnover rate in the initial phase

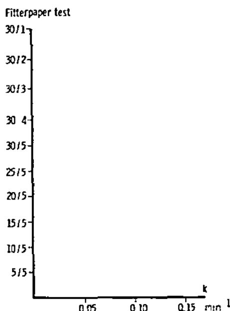


Fig 12
relation between filterpaper test and fractional turnover rate in the basal pha e

id not turned to either side (Figs 6A & 6B) or turned to the wrong side the hnetium pooled in the outer canthal area resulting in irregular shaped curves n the studies with closed eyes the persons were asked to blink frequently for out one min to blink out the surplus volume after installation A diphasic ipe with a change of the fractional turnover rate at 5–6 min as in open eyes s seen in three persons (note change of elimination curve in Fig 8 after min) In six persons no such change was seen after 5–6 min (Fig 7) The an fractional turnover rate in these nine persons was found to be 0 048 ± 011 min⁻¹ mean ± SEM (using the basal phase from 7 to 15 min in the three rsons with a diphasic shape) corresponding to a tear flow of 0 34 µl min ¹ nch is hi hly significant from tear flow in open eyes (t test 2 $P < 0.001$) In 5 of the 9 persons with closed eyes intermittent phases (rapid elimination ase in Table III) with a higher elimination rate were seen (in the person in g 7 from 23 to 26 min after instillation) The mean fractional turnover rate these phases was found to be 0 189 ± 0 020 min ¹ (mean ± SEM n = 5) The ifference between the rapid elimination phases and the basal phases in the osed eye situation was significant (t test 2 $P < 0.001$) In four persons rapid limination phases were less pronounced or absent (in the person in Fig 8 a mall rapid elimination phase is placed from 9 to 10 min after instillation)

Tear flow calculations beyond the 15 min measurin time was not car out in persons with open eyes because the fractional turnover rates after time approximated the values found after irrigation of the co junctiva min^{-1} Sorensen & Taagehoj Jensen 1977) The phases with rapid el were found in the time interval 4–30 min after instillation For tech reasons studies beyond 35 minutes have not been carried out.

The background corrections were calculated by the computer To facil corrections in later studies and to illustrate the magnitude of the back corrections a nomogram was constructed using equations (1) and (?) (Fig There are three variables in these calculations The natural backgro percent of A_0 $\frac{B_n}{A_o}$ %) the background due to absorption and redistribu the radioisotope ($\frac{B_l}{A_o}$ %) and the fractional turnover rate $\frac{B_n}{A_o}$ was f to be 2 1 % ± 0 3 % (mean ± SEM) and $\frac{B_l}{A_o}$ % to be 6 8 % ± 1 0 % (me SEM) The fractional turnover rates in the initial phases were not correc background radiation since $\frac{B_n}{A_o}$ and $\frac{B_l}{A_o}$ were very small in this pha the elimination

In Fig 10 is shown an example of the curves used in the correction Th basal phase in Fig 3 has been used due to its high background corr Curve b is the corrected curve when the example is from a single or f determination (b = a – d) and curve c is the corrected curve when the exam is from a second determination 5 min after the end of the firs d termina (c = a – e)

The results of the filterpaper readings can be seen in Figs 11 & 12 Neit in the initial nor the basal phase was a correlation to the fractional turnov rate demonstrated

Discussion

The fractional turnover rate can be used as a parameter for tear flow The te flow L (µl min^{-1}) can be calculated from L = k V where V is the te r vol in the conjunctival sac

The tear volume has been determined by Mishima et al. (1966) to whereas Zintz & Schilling (1964) found a value of 28 µl in young and µl old individuals For reasons mentioned by Mishima et al a tear volume 7 µl seems most reasonable Furthermore they found that the tear volume

slightly dependent on tear flow (a ten fold increase in tear flow caused a ling of the tear volume)

ie amount of transport of the radioisotope through the conjunctiva to the ral circulation also influence the calculation of tear flow Sørensen & ehoj Jensen (1979) found a transport of 1 5% min $^{-1}$ of technetium per ietate in normal human eyes Whether this transport is accompanied by a r transport of the same magnitude is not known If a water transport takes e the tear flow calculation needs no correction for the transconjunctival sport of technetium If water is not transported the mean fractional turn rate should be corrected from 0 083 min $^{-1}$ to 0 068 min^{-1} and the tear from 0 6 μl min^{-1} to 0 5 μl min $^{-1}$ (corrected for the half life of techne ı = 6 h)

he evaporation from the precorneal tear film has been found to 0 1 μl $^{-1}$ in rabbits (Iwata et al 1969 Mishima & Maurice 1961) correcting the flow to 0 7 and 0 6 μl min $^{-1}$ respectively

he difference between first and second determination was less pronounced he basal phase One explanation can be that the approximated background rection in the second determination was not high enough The other ex nation is that patient was more quiet during the second determination re ting in less reflex lacrimation Consequently one would expect greatest dif ence in the initial phases which in fact is the case

The interpretation of the diphasic shape of the curves has been that the tillation of technetium caused some stimulation to the eye resulting in an reased tear flow for approximately five min This initial higher fractional nover rate was not as high as that caused by the intense stimulation from a terpaper in the fellow eye

No difference was found between the fractional turnover rates in the supine d uprı ht position During the measurements the patients heads were turned proximately 20 degrees to the side opposite to the one being investigated If is tilting of the head was omitted a pooling of the radioactivity at the outer nthal area was seen on the oscilloscope display resulting in irregular shaped irves and often an overflow out of the lateral canthus The advantage of per rming the investigation in the supine position was the steady fixation of the ad which was not easily obtained in the upright position for 15 min

Differences between the erect and supine position was found by Hurwitz t al (1975) on seven normal persons The authors did however apparently ot tilt the patients head and the difference is therefore understandable urthermore their region of interest was the inner canthus – not the whole onjunctival sac Generally the gravitational force is considered of some im ortance in tear drainage This assumption seems thus only valid when the

outer canthus is placed at the same level or lover than the [illegible] accordance with the observation made by Maurice (19 3) [illegible] a drop of 10 μl injected into the eye will fall to the outer canthus [illegible] there dispite strong continuous blinking Figs 6A & 6B show the [illegible] radioactivity at the outer canthal area

The explanation of the intermittent high fractional turnover [illegible] eyes is not obvious An intermittently high tear secretion would not [illegible] a high fractional turnover rate without a simultaneous rapid drain[illegible] secreted tears Therefore intermittently high outflow must take place [illegible] closed eye situation in some persons with or without a simultaneously [illegible] secretion The rapid outflow can not be explained by a few blinks [illegible] rapid outflow continued for 1–5 min.

The filterpaper readings showed that the Schirmer test apparently [illegible] value in normal persons

Mishima et al (1966) found a tear flow twice as big as in this study [illegible] region of interest was a small area in the lower marginal tear strip [illegible] our region of interest was the entire conjunctival sac. By Mishima et [illegible] tion method a correction for fluorescein diffusion to the general [illegible] was not calculated whereas the present method yielded a tear flow [illegible] pensated for the conjunctival transport of technetium

Acknowledgment

The study was supported by the Bausch & Lomb Company

References

Balik J (1952) The lacrimal fluid in keratoconjunctivitis sicca. *Amer J Oph* [illegible] 7 3–752

Brandt H P & Fritsche B (1967) Klinische Erfahrungen mit dem Tranen[illegible] verdunnungstest von M S Norn. *Acta ophthal (Kbh)* 45 [illegible]

Carlton W H Trueblood J H & Rossomondo R. M (19 3) Clinical evalu[illegible] microscintigraphy of the lacrimal drainage apparatus *J nucl Med* 14 [illegible]

Dressler J & Denffer H V (1974) Erste Erfahrungen mit der Funktionsun[illegible] der Tranenwege *Radioaktive Isotope in Klinik und Forschung* 11 [illegible]

Dressler J & Denffer H v (1975) Vergleichende Untersuchungen zum [illegible] Verhalten von ^{99m}Tc Pertechnetat ^{99m}Tc Humanalbumin Mikrosphären und [illegible] Schwefelkolloid in der Tranenflussigkeit In Pabst H W Hor G [illegible] H A E eds *Nuklearmedizin* pp 822–826 F K Schattauer Verlag Stuttgart- [illegible] York.

Ehlers N (1967) A calculation of the average tear flow *Acta ophthal (Kbh)* 45 274

-rg G P & Berens C (1961) A standardized Schirmer tear test kit *Amer J thal 51* 840–84?

erger R Hanna C. & Boyd C M (19 5) Effects of drug vehicles on ocular con time *Arch Ophthal (Chicago) 93* 4?–45

itz J J Maisey M N & Welham R A N (19 5) Quantitative lacrimal scintil aphy I Method and physiological application *Brit J Ophthal 59* 30?–312

S Lemp M A Holly F J & Dohlmann C H (1969) Evaporation rate of water n the precorneal tear film and cornea in the rabbit *Invest Ophthal 8* 613–619

ner C (1964) Untersuchungen uber das Ausmass der Tranensecretion beim Men en *Klin Mbl Augenheilk 144* 412–41?

rce D M (1973) The dynamics and drainage of tears *In Ophthal Clin 13* –116

r P B & Dausch D (1975) Klinische Erfahrungen mit der Radionuklik Dakryo tographie. *Klin Mbl Augenheilk 16?* 4?1–4?6

ima S & Maurice D M (1961) The oily layer of the tear film and evaporation m the corneal surface. *Exp Eye Res 1* 39–45

ima S Gasset A Kl?ce S D & Baum J L (1966) Determination of tear volume d tear flow *Invest Ophthal 5* 264–2?6

Y (19 6) Studies of tear flow using sodium pertechnetate Tc 99M *Jap J hthal 90* ?83–?89

M S (1965) Tear secretion in normal eyes *Acta ophthal (Kbh) 43* 56?–573

A & Jaeger W (195?) Kolorimetrische Methode zur Messung der Tranen cretion *Klin Mbl Augenheilk 121* 419–425

omondo R M Carlton W H Trueblood J H & Thomas R P (1972) A new thod of evaluating lacrimal drainage *Arch Ophthal (Chicago) 88* 5?3–5?5

rmer O (1903) Studien zur Physiologie und Pathologie der Tranenabsonderung d Tranenabfuhr *von Graefes Arch Ophthal 55* 197–?91

nsen T & Taagehøj Jensen F (19 5) Determination of tear flow using a radio tive tracer *Acta ophthal (Kbh) Suppl 1?5* 43–44

nsen T & Taa ehøj Jensen F (19 ?) Methodological aspects of tear flow deter ination by means of a radioactive tracer *Acta ophthal (Kbh) 55* ?26–737

nsen T & Taagehøj Jensen F (19?9) Conjunctival transport of technetium – 99m rtechnetate *Acta ophthal (Kbh) 57* 691–699

z v R & Schilling T (1964) Ein kolorimetrisches Verfahren zur Messung des lussigkeitsvolumen im Bindehautsack *Klin Mbl Augenheilk 144* 393–412

Ho s address

ørensen,
artment of Ophthalmology
rus Kommunehospital
8000 Århus C
nmark.

Department of Ophthalmology (Head Sven Erik G Nilsson),
University of Linköping Sweden

PHAGOCYTOSIS OF LATEX MICROSPHERES BY THE EPITHELIAL CELLS OF THE GUINEA PIG CONJUNCTIVA

BY

STEFAN LATKOVIC and SVEN ERIK G NILSSON

The capability of conjunctival epithelial cells to phagocytize particulate foreign matter was studied experimentally in the guinea pig Perumbal conjunctival epithelium was prepared for transmission electron microscopy by perfusion fixation 4 and 24 h after instillation of latex microspheres (0 79 μ in diameter) into the lower conjunctival fornix A varying number of microspheres were internalized by the superficial epithelial cells in both cases The intracellular microspheres single (at 4 and 24 h) or aggregated (only at 24 h) were always membrane enclosed corresponding to primary phagosomes Some indications of secondary phagosomes were seen as well In the 24 h specimens microspheres were present also in the intermediate epithelial cells implying a transfer from superficial to deeper cells Thus superficial as well as intermediate epithelial cells of the guinea pig conjunctiva are capable of active phagocytosis of inert particles

Key words conjunctival epithelium – phagocytosis – latex microspheres – transmission electron microscopy

The polymorphonuclear leukocytes and the mononuclear phagocytes are [illegible] nerally regarded as the professional phagocytizing cells (Stossel 19[illegible] [illegible] number of recent studies demonstrated that other types of cells not cha[illegible] terized as phagocytes are capable of internalizing bacteria or particulate [illegible] ter by a process resembling phagocytosis epidermal keratinocytes (Wo[illegible] Konrad 1972) retinal pigment epithelium (Hollyfield & Ward 19[illegible] Fe[illegible] & Mixon 1976) epitheloid HeLa cells (Bovallius & Nilsson 1975 Kihlstr[illegible]

Received February 1 1979

ʒvic 1978) muscle cells (Garfield et al 1975) and mucosa of the genital (Ward et al 1975) Intracellularly located bacteria were also observed in inctival epithelial cells (Racz & Tenner 1963 Tenner et al 1971 Zimian t al 1974) and the process was regarded as endocytosis/phagocytosis
the case of bacteria found within the epithelial cells opinions differ as to process by which the bacteria pass across the cell membrane While some ors regard it as phagocytosis (Zimianski et al 1974 Bovallius & Nilsson Ward et al 1975 Kihlstrom & Latkovic 1978) others consider it as nal penetration through the cell membrane (Takeuchi & Sprinz 1967 uchi et al 1968 Takeuchi 1975 Ward et al 1974 Gemski & Formal)
ne present study was undertaken to determine whether conjunctival epi al (CE) cells are capable of phagocytosis Using latex microspheres a metabolic, non toxic tracer instead of biological material it was possible inimize the influence of the material to be phagocytized and thus in a e simplified system study the responses of the phagocytizing cells
or the ultrastructure of the normal perilimbal conjunctival epithelium of guinea pig the reader is referred to Latkovic & Nilsson (1979a b)

Material and Methods

o clinically healthy adult pigmented guinea pigs were anaesthetized intra itoneally with 20 mg/kg b w sodium pentobarbital (Nembutal® Abbott) l the eyelids closed with agraffes Latex microspheres 0 794 μ in diameter plied in a 10 % suspension (weight/volume) (Dow Chemical) were intro ed into the lower conjunctival fornix of each eye by instilling approxi tely 0 05 ml through a needle with a rounded point After 4 h one of the mals was perfused via the left ventricle with 4 % glutaraldehyde in 0 1 M lium cacodylate buffer at pH 7 2 Each eye was removed in one piece to her with the eyelids and fixation was continued for 72 h by immersion in taraldehyde solution as above but at 4 C The second animal was kept htly anaesthetized by repeated small doses of sodium pentobarbital Every h 0 05 ml of latex suspension was instilled into the lower fornix of each eye above After 24 h the second animal was perfused and the eyes excised and ation performed as above Parallel sided strips of conjunctiva from the nbus to the margin of the lower eyelid were dehydrated in increasing con ntrations of ethanol and embedded in Epon Thin sections of conjunctival ithelium from the perilimbal zone were cut at right angles to the limbus r further details see Latkovic & Nilsson (1979a)



Fig 1

Survey electron micrograph of guinea pig conjunctival epithelium from the per
zone slightly obliquely cut demonstrating latex microspheres (L) as single or
in aggregates in polyhedral (P) cells of the intermediate layers M melan
N nucleus S superficial cell VA vacuole (24 h incubation with latex particles



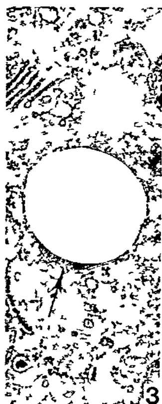


Fig 2

…mary phagosome with a single latex microsphere (L) in the apical part of a superficial … An autophagic vacuole is seen to the left of the microsphere VE vesicles (4 h incubation) × 4? 600

Fig 3

…ofile of what may be a secondary phagosome containing a single latex microsphere …d some electron dense material (arrow) within the membrane (4 h incubation) × 43 300

Observations

…rofiles of latex microspheres very low in electron density rather uniform in …ze and round or slightly oval in shape were easily identified (Fig 1) The …tex substance of the microsphere was extracted to a large extent but a re…due was often seen as an irregular area of somewhat higher electron density …igs 1 2 and 4)

The CF cells showed normal ultrastructural characteristics without any signs …f damage.



Fig 4

Two secondary phagosomes in the perinuclear zone of an epithelial cell Two and latex microspheres (L) respectively are visible in the section. Arrows point at the surrounding membrane Note the granulated electron dense material between and around the microspheres N nucleus MI mitochondria VA vacuole (24 h incubation) ×

No latex microspheres were observed attached *to* the free surface of the epithelium The number of *microsphere* containing cells per section and the number of *microspheres* per cell *varied within* a wide range in both experiments

After 4 h incubation with latex suspension the microspheres were found only in the superficial CE cells They were observed as single units at various levels within the cell The most *superficial microspheres* were located approximately one microsphere diameter *beneath the free surface* (Fig 2) while the deeper ones were most often seen in the perinuclear area All microspheres

bound by a membrane usually tightly fitting but sometimes with narrow spaces which in certain cases could be filled with an electron dense ma (Fig 3)

the eyes subjected to fixation 24 h after introduction of latex suspension e inferior fornix the distribution of microspheres within the cells was rather rent The number of microspheres in the superficial cells was fewer com d to the 4 h experiment and they were observed even in the polyhedral of the intermediate layers Both in the superficial and the polyhedral CE the microspheres were found either as single units or as small aggrega s (Figs 1 and 4) In addition to microspheres in different numbers the ibrane sometimes also enclosed finely granulated material (Fig 4) or small cles Melanosomes could also be included in the aggregates (Fig 1) Al igh microspheres were seen in the apical cell compartment most of them concentrated in the perinuclear zone especially in the polyhedral inter liate cells and not infrequently in the vicinity of nuclear indentations s 1 and 4)

Discussion

phagocytic capability of CE cells was suggested in studies with different ains of bacteria (Racz & Tenner 1963 Tenner et al 1971 Zimianski et al 4) The results of these investigations were not quite conclusive since the de of bacterial entry into the cells could be either active penetration or ocytosis

The present study was undertaken to de ermine whether unstimulated CE lls are capable of phagocytosis The possible influence on the phagocytizing lls of the material presented for phagocytosis was minimized by using micro heres of latex a non metabolic substanse which was found to be non toxic the host cells in a number of *in vitro* and *in vivo* studies (Schoenberg et al 61 Korn & Weisman 1967 Wolff & Konrad 1972 Garfield et al 1975 ollyfield 1976 Feeney & Mixon 1976) Hollyfield & Ward (1974) however ported a toxic effect of latex microspheres on the retinal pigment epithelium f the *Rana pipiens* embryo but not of the tadpole or of the frog Latex micro heres are easily identified in the electron microscope

Attachment of particles to the cell membrane initiates the subsequent stages f internalization (Simson & Spicer 1973) Since latex microspheres were ob erved within CE cells it can be deduced that they had been attached to the ell membrane prior to uptake The fact that no microspheres were found at ached to the free cell surface in our material may be explained by dissolution

Simson J V & Spicer S S (1973) Activities of specific cell constituents in ph[illegible] (endocytosis) *Int Rev exp Path 12* 79–118

Stossel T P (1974) Phagocytosis *New Engl J Med* 290 71[illegible]

Takeuchi A (1975) Electron microscope observations on penetration of the [illegible] thelial barrier by *Salmonella typhimurium* In Schlessinger D Ed Mi[illegible] 1975 American Society for Microbiology Washington D C

Takeuchi A & Sprinz H (1967) Electron microscope studies of experimental [illegible] infection in the preconditioned guinea pig II Response of the intestinal m[illegible] the invasion by *Salmonella typhimurium Am J Path 51* 13[illegible]–161

Takeuchi A Formal S B & Sprinz H (1968) Experimental acute colitis in the [illegible] monkey following peroral infection with Shigella flexneri *Am J Path* [illegible]

Terner C Racz P & Redey B (1971) Significance of the epithelial phase in [illegible] mental salmonella conjunctivitis *Acta Microbiol Acad Sci Hung 18* 16[illegible]

Ward M E Watt P J & Robertson J N (1974) The human Fallopian tube [illegible] ratory model for gonococcal infection *J Infect Dis 129* 6[illegible]

Ward M E Robertson J N Englefield P M & Watt P J (1975) Gonococcal [illegible] tion invasion of the mucosal surfaces of the genital tract In Schlessinger D [illegible] *Microbiology – 1975* American Society for Microbiology Washington D C

Wolff K & Konrad K (1972) Phagocytosis of latex beads by epidermal kera[illegible] *in vivo J Ultrastruct Res 39* 262–280

Zimianski M C Dawson C R & Togni B (1974) Epithelial cell ph[illegible] *Listeria monocytogenes* in the conjunctiva *Invest Ophthal 13* 6[illegible]

Authors address

Dr Stefan Latkovic and Prof Sven Erik G Nilsson
Department of Ophthalmology
University of Linkoping
S 581 85 Linkoping
Sweden

ment of Ophthalmology (Head Sven Erik G Nilsson) Linkoping University and Departments of Clinical Neurophysiology (Head Lennart W iden) Neurology (Head Eric Kugelberg) Karolinska Hospital Stockholm Sweden

CONTRACTILE PROPERTIES OF EXTRACULAR CLE IN CATS REARED WITH MONOCULAR LID CLOSURE AND ARTIFICIAL SQUINT

BY

GUNNAR LENNERSTRAND AND JERKER HANSON

ittens were raised with amblyopia by monocular lid suture or esotropia y transection of extraocular muscles in one eye at the age of 2–3 weeks sometric contractions in the inferior oblique muscle were recorded at 20 veeks or at adult age and the results were compared with those of sim larly aged cats with normal visual development The speed of contraction nd the fatigue resistance was reduced in the lid sutured animals In the sotropic cats studied fatigue resistance was also reduced but the speed f contraction did not change much

It is suggested that the postnatal eye muscle development can be modified by manipulation of the visual input at an early age Presumably the im paired binocular vision in the lid sutured and esotropic cats reduced the demand for fusion vergences and this might be reflected in the changes of eye muscle properties In monocularly lid sutured cats eye muscle hanges were the same on both sides suggesting that amblyopia *per se* did not affect eye muscle development

Key words Extraocular muscle – cats – contractile properties – mono lateral lid suture – artificial squint – binocular deficiency

l functions of most mammals are subnormal at birth and are subject to a postnatal improvement In the cat visual acuity and binocular vision o have reached maximal capacity at the age of 10 to 20 weeks the period most rapid change occuring between 3 and 6 weeks of age (Blakemore Sluyters 1975 Mithchell et al 1976) Oculomotor functions employed for

ed January 29 1979

fixation and binocular vision are probably fully developed ... age as indicated by a study of the postnatal eye muscle development ... kitten (Lennerstrand & Hanson 1978 *a b*)

Numerous investigators have shown that the binocular functions of cats ... be severely impaired by monocular deprivation or artificial squint ... before or during the most sensitive period of visual development, i.e. ... of 3–6 weeks (see Blakemore 1977) It has not been demonstrated ... whether the binocular defects are accompanied by any changes ... properties We have compared contractile properties of inferior oblique ... from normal cats and cats with monocular lid suture and artificial squint ... the speed of muscle contraction and the fatigue properties seemed to be ... by the operations which were performed before the sensitive period ... previously

The results have been reported elsewhere in a preliminary form (Lennerstrand & Hanson 1978 *c*)

Methods

The experiments were performed on four normal cats and ten cats from ... litters with impaired binocular vision These cats were raised in the same ... Additional data have been obtained from two adult, normal cats, also ... study of normal eye muscle development (Lennerstrand & Hanson 1978 ...) ... operations on the cats with binocular deficiencies were performed at the age ... weeks Under ketamine anaesthesia either (1) the left eye was ... closed ... ing first the nictitating membrane to the upper fornix and then the lids ... or (2) a left sided convergent squint was produced by transsection of the ... muscle and the lateral slips of the retractur bulbi muscle of the left eye ... necessary repair of the lid closure was done within one day One group of ... consisting of two normal one esotropic and two lid sutured cats were examined ... age of 70 weeks Another group of four normal two strabismic and ... cats were examined when they were adult (11–15 months of age) Of the ... muscles sectioned only those with clear esotropia (convergent squint) were ... physiologically The experiments were performed on the inferior oblique ... unoperated right eye in strabismic cats the surgical trauma might have ... *ferior oblique function on the operated side In lid sutured and normal cats* ... on both sides were used

The experimental technique has been described in detail in previous papers ... & Lennerstrand 1977 Lennerstrand & Hanson 1978 *a b*) Isometric tension ... were made with the muscle attached to a sensitive force measuring system ... element being a semiconduction tranducer (Pixie 8101 Endevco Laboratories ...) ... muscle was activated by nerve stimulation with bipolar platinum electrodes ... pulses of 0 1 msec duration were delivered singly or in trains from a Grass S... ... tor Twitch characteristics tetanic tension development, fatigue properties and ...

behaviour were studied in the same manner as previously described. All measurements were done with the muscle a optimal lengh for twitch con

hysiological results from the two eyes of the same animal were compared with statistics in normal cats and lid sutured animals Data from normal and lid cats were compared with t statistics for two means. The amount of data on c cats in each age group was too small to be statistically assessed.

Results

e contractile characteristics of inferior oblique muscles of the two eyes ompared in lid sutured cats and in normal animals No statistically ant differences were found within each group for any of the parameters This held true both for the kittens 20 weeks of age and the adult cats indings justified that data from both eyes were lumped in the com between normal and lid sutured cats The differences in contractile ies between muscles of normal lid sutured and esotropic cats have been ized in Table I For all other contractile properties studied the values e same in normal and operated animals It was found that in adult cats contraction time (*ct*) and half relaxtion time (*hrt*) were longer in lid than in normal animals i e the muscles of adult lid sutured cats con slower than the normal adult eye muscles (Fig 1 and Table I) There to be very little difference in this respect between adult esotropic cats ılt normal cats, or between 20 weeks old lid operated and normal ani Table I) The maximal rate of tension rise during tetanic contraction was r in lid sutured than in normal animals of the adult age but this dif was not statistically significant. Maximal tension in twitch contraction was the same in all groups of the same age

difference between the normal and the lid operated animals were ered in the fatigue properties of the muscles As shown in Fig 2 the drop on during continuous activation for 30 sec was faster and deeper for than for normal animals i e. the muscles of lid sutured animals higher susceptibility to fatigue than muscles of normal animals both at s and at adult age The differences were statistically significant for s frequencies of 50 and 100 Hz (Table I) but not for 200 Hz The same for the two adult esotropic cats studied

ecovery of the twitch response from a 30 sec fatiguing stimulation was ower in the lid operated adult animals than in adult normal cats This prominent in the 100 Hz and 200 Hz stimulations The depression of amplitude was more profound and lasted longer in operated than in

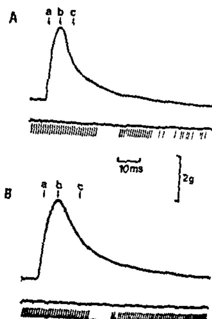


Fig 1

Isometric twitch responses (top traces) to supramaximal nerve stimulation in a [illegible] with normal binocularity (A) and with monocular lid suture (B) Arrows indicate [illegible] (a) peak (b) and point of half relaxation (c) of the twitch contraction The [illegible] traction time (*ct*) is time interval a–b and half relaxation time (*hrt*) interval [illegible]
Lower traces for stimulus and time marking

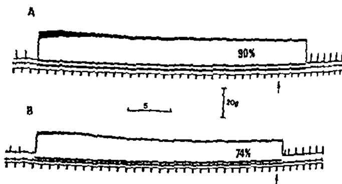


Fig 2

Endurance of adult inferior oblique muscles in a normal (*A*) and a monocular [illegible] sutured cat (*B*) Top traces show muscle tension to the extreme left and right [illegible] responses interrupted by a period of continous 100 Hz stimulation. Fatigue [illegible] has been measured as the amount of tension in per cent of the initial tetanic tension remaining after 30 sec (arrow) The fatigue resistance is higher in the normal [illegible] than in the lid sutured cat (74%)
Middle and bottom traces used for stimulus and time marking respectively

Twitch contraction time (ct) and half relaxation time (hrt) were measured from responses to single pulse stimulations as shown in Fig 1. Endurance was assessed as shown in Fig 2.
Post tetanic twitch amplitude and hrt were determined for twitches obtained 5 min after a 90 sec continuous tetanization at the rates of stimulation shown. These values have been given in percent of the corresponding pre tetanic value.

	20 weeks			*Adult*			
	N (n = 4)	I (n = 4)	P	N (n = 8)	L (n = 10)	P	E (n = 2)
Twitch							
ct (ms)	7 0 ± 0 3	7 2 ± 0 9	–	5 7 ± 0 6	6 8 ± 1 0	**	5 9
hrt (ms)	11 4 ± 2 3	9 2 ± 1 7	–	7 0 ± 1 0	9 6 ± 2 6	*	8 0
Endurance (%)							
at 50 Hz	138 ± 26	96 ± 20	*	170 ± 35	112 ± 22	**	106
at 100 Hz	79 ± 7	57 ± 15	*	95 ± 15	50 ± 8	*	84
Posttetanic twitch 100 Hz							
ampl (%)	81 ± 7	81 ± 4	–	82 ± 15	70 ± 6	*	72
hrt (%)	145 ± 22	120 ± 12	–	102 ± 10	113 ± 16	*	123
Posttetanic twitch 200 Hz							
ampl (%)	84 ± 8	90 ± 2 6	–	59 ± 9	62 ± 8	***	78
hrt (%)	185 ± 44	154 ± 34	–	105 ± 22	149 ± 46	*	135

– $P > 0\ 05$ * $0\ 05 > P > 0\ 02$ ** $0\ 02 > P > 0\ 01$ *** $P < 0\ 01$

normal animals and the changes in *hrt* were more marked in operated [illegible] shown in Table I for values obtained 5 min after the fatiguing stimuli [illegible] the other hand the same time course of recovery was seen in both lid [illegible] and normal cats for the tetanic response to 200 Hz for 0 5 sec The [illegible] ency applied to results from esotropic cats In animals 90 weeks of a[illegible] and tetanic recovery was the same for normal and operated groups

Discussion

This study has shown that manipulation of the visual input for kittens aff[illegible] not only the visual but also the oculomotor development Monolateral lid s[illegible] and acquired squint at an early age have been shown to change the prop[illegible] of neurons in the visual cortex (see e g Blakemore 1977)

However it has not been previously known that these procedures can [illegible] changes in the speed of twitch contraction or the fatigue resistance of [illegible] muscles The cats with monolateral visual deprivation due to lid suture sh[illegible] eye muscle changes of the same degree in the seeing and the closed eye Th[illegible] it seems unlikely that the variations in eye muscle properties between [illegible] and lid sutured cats were caused by monocular amblyopia Instead it w[illegible] appear that the changes seen in operated cats were in some way correl[illegible] the state of their binocular vision since the eye muscle changes seemed [illegible] marked in lid sutured cats than in the squinting cats

Both the speed of contraction and the fatigue resistance were affected in th[illegible] lid sutured animals but only fatigue properties in esotropic cats B[illegible] vision is completely impeded in animals with monocular lid suture w[illegible] esotropic cats probably some peripheral binocularity remained The ce[illegible] were considered normal with regard to binocular vision The conclusions co[illegible] cerning binocularity were inferred from data obtained in studies by ot[illegible] investigators on the visual cortex of normal cats (Hubel & Wiesel 196[illegible] B[illegible] 1973) of cats with monocular visual deprivation (Hubel & Wiesel 1970 Bl[illegible] more & van Sluyters 1974) and of cats with acquired esotropia (Ikeda 19[illegible]

If binocular vision is defective or lacking the demand for fusion verg[illegible] eye movements is probably very much reduced It may be speculated tha[illegible] decreased demand affected the development of eye muscle fibers leading to [illegible] changes seen in eye muscle properties The slow fiber system (Hess & P[illegible] 1963 Matyushkin 1964 Bach y Rita & Ito 1966) presumable responsible f[illegible] slow motor acts like fusion vergences (Lennerstrand 1975) would seem p[illegible] ferentially engaged The lower fatigue resistance of eye muscles in oper[illegible] animals of all ages tested would support this idea Earlier studies indicated th[illegible]

v fiber system developed earlier than the fast fiber system (Lenner & Hanson 1978 a b) and that it had reached a high degree of maturity cats during the so called sensitive period of the visual development very precise control of eye mobility is needed However the speed of on was lowered in adult cats with lid suture but not in 20 weeks old iggesting that also the fast fiber system evolved differently in the later f development

ight be of interest to compare the changes in eye muscle properties with d in fast and slow skeletal muscle by disuse although the methods ization of a limb or denervation of the muscles used in the skeletal studies would seem to limit muscle function much more drastically than ed selective abolition of fusion vergences in this study It should also in mind that eye muscles have a specialized fiber composition with ng in skeletal muscle (Alvarado & van Horn 1975) which a direct comparison with other muscles difficult As a result of ation adult cat slow and fast hind limb muscles show reduced speed of ion (Syrovy et al 1972 Kean et al 1974) Prolonged inactivity induced e changes in cat muscles (Davis & Montgomery 1977) Immobilization t lead to any significant changes in the speed of contraction in hind iuscles of the guinea pig (Maier et al 1976) The present experimental ı with a proposed loss of fusion vergences may be considered equivalent irtial denervation but not to an immobilization Disuse of certain eye components may explain the reduction in speed of contraction that oc after monocular lid suture It is also well known that disuse lowers the potential and the fatigue resistance of a muscle (Hooloszy & Booth and this might have occurred also in the eye muscles of lid sutured and ic animals In new born animals disuse by denervation prevents the muscle fiber differentiation (Karpati & Engel 1967 Shafiq et al 1972) lan to expand the investigation by examining the same eye muscles with mical techniques and compare fiber composition and enzymatic profiles cles in normal and binocularly impaired cats

present experimental technique has offered unique possibilities to study ects on muscle development of a limited but well defined change in nal demands on a motor system Thus it has been shown that disuse of oculomotor functions in this case probably restricted to fusional ces may induce significant changes in eye muscle properties From a standpoint the results may prove of importance for clinical research nt in humans If the muscle changes induced by loss of binocular vision ble the lack of the muscular substrate for fusion vergences might proper eye alignment impossible in spite of a sensory cure through c treatment

Acknowledgment

This study was supported by grants from Karolinska institutets fonder ... Committee of Östergotlands lans landsting and the Medical Research Council, Sweden (no 04X–4751)

References

Alvarado J & van Horn C (1975) Muscle cell types of the cat inferior ...
Lennerstrand G & Bach y Rita P Eds *Basic Mechanisms of Ocular Motility and their Clinical Implications* pp 15–43 Pergamon Press Oxford
Bach y Rita P & Ito F (1966) In vivo studies on fast and slow muscle fibers ... extraocular muscles *J gen Physiol 49* 1177–1198
Bishop P O (1973) Neurophysiology of binocular single vision and stereopsis. In Jung R Ed *Handbook of Sensory Physiology* vol VII/3 Part A pp ... Springer Verlag Berlin.
Blakemore C (1976) Modification of visual function by early visual experience ... *schuet Akad med Wiss 32* 13–28
Blakemore C & van Sluyters R C (1974) Reversal of the physiological effects of monocular deprivation in kittens further evidence for a sensitive period *J Physiol (Lond) 237* 195–216
Blakemore C & van Sluyters R C (1975) Innate and environmental factors in the development of the kitten s visual cortex *J Physiol (Lond) 248* 663–716
Davis C J F & Montgomery A (1977) The effect of prolonged inactivity ... contraction characteristics of fast and slow mammalian twitch muscle *J Physiol (Lond) 270* 581–594
Hess A & Pilar G (1963) Slow fibers in the extraocular muscles of the cat *J Physiol (Lond) 169* 780–798
Holloszy J O & Booth F W (1976) Biochemical adaptation to endurance exercise in muscle *Ann Rev Physiol 38* 273–291
Hubel D & Wiesel T (1962) Receptive fields binocular interaction and functional architecture in the cat s visual cortex *J Physiol (Lond) 160* 106–154
Hubel D & Wiesel T (1970) The period of susceptibility of the physiological effects of unilateral eye closure in kittens *J Physiol (Lond) 206* 419–436
Hanson J & Lennerstrand G (1977) Contractile and histochemical properties of the inferior oblique muscle in the rat and in the cat *Acta Ophthal (Kbh)* ...
Karpati G & Engel W K (1967) Neuronal trophic function *Arch Neurol* ...
Kean C J C Lewis D M & McGarrick J D (1974) Dynamic properties of denervated fast and slow twitch muscle of the cat *J Physiol (Lond) 2*... 103–113
Lennerstrand G (1975) Motor units in eye muscles In Lennerstrand G & Bach y Rita P Eds *Basic Mechanisms of Ocular Motility and their Clinical Implications* pp 119–143 Pergamon Press Oxford
Lennerstrand G & Hanson J (1978*a*) The postnatal development of the inferior oblique muscle of the cat I Isometric twitch and tetanic properties *Acta physiol scand 103* 132–143
Lennerstrand G & Hanson J (1978*b*) The postnatal development of the inferior oblique muscle of the cat II Effects of repetitive stimulation on isometric twitch response *Acta physiol scand 103* 144–153

G & Hanson J (1978*c*) Effects on cat eye muscle function of impaired ular vision *Acta Ophthal (Kbh)* 56 478–479
Crockett J L Simpson D R Saubert IV C W & Edgerton V R (1976) s of immobilized guinea pig hind limb muscles *Amer J Physiol 231* 576
hkin D P (1964) Motor systems in the oculomotor apparatus of higher animals *Proc (Transl suppl) 23* 1103–1106
I D E Giffin F Wilkinson F Anderson P & Smith M L (1976) Visual ution in young kittens *Vision Res 16* 363–366
S A Asiedu S A & Milhorat A T (1979) Effect of neonatal neurectomy on rentiation of fiber types in rat skeletal muscle *Exp Neurol 35* 529–540
I Gutmann E & Melichna J (1972) The effect of denervation on contraction myosin properties of fast and slow rabbit and cat muscles *Physiol Bohemoslov* 53–359
P (1976) Age dependence of the effect of squint on cells in kittens visual cortex *Brain Res 97* 151–157

s address
nnar Lennerstrand
ment of Ophthalmology
ty Hospital
5 *Linkoping*

Department of Ophthalmology (Head Salme Vannas)
University of Helsinki Finland

A CLINICAL STUDY ON THE DETECTION OF STRABISMUS ANISOMETROPIA OR AMETROPIA OF CHILDREN BY SIMULTANEOUS PHOTOGRAPHY OF THE CORNEAL AND THE FUNDUS REFLEXES

BY

KARI KAAKINEN and VEIKKO TOMMILA

Twenty two strabismus and 106 straight eyed patients with anatomically normal eyes were first photographed with a conventional camera equipped with a weak 100 mm teleobjective and coaxial flashlight and then examined clinically The possibility of detecting strabismus anisometropias and ametropias in the photographs by noting the localisation of the corneal reflexes and examining the appearance and lightness of the fundus reflexes and their possible asymmetry were tested in a double blind study Even small angled strabismus cases could be found because of the asymmetrical localisation of the corneal reflexes In 18 of the 22 strabismus cases (82 %) there was asymmetrical lightness of the fundus reflexes and the fundus reflex of the deviating eye was lighter than that of the fixating eye All the straight eyed anisometropias of 3 0 diopters or more (five cases) were observed in the photographs because of the asymmetrical appearance of the fundus reflexes In straight eyed anisometropias of under 3 0 diopters the fundus reflexes were symmetrical in 90 cases and asymmetrical in 11 cases (11 %)
Only three out of eight hyperopias of from + 4 5 to + 6 0 diopters were found because of the light crescent in the low part of the pupil All myopias of over − 4 0 diopters (14 cases) were observed because of the light crescent appearance in the upper part of the pupil No pupillary crescents appeared with refractions of less than − 1 5 diopters myopia or less than + 4 5 diopters hyperopia 179 eyes came within this range
Even a technician can perform without premedication the method tested here for rapid and simple screening to detect *strabismus* and straight eyed

Received December 11 1978

anisometropias of 3 0 diopters or more in small children or other patients who do not co operate well in normal clinical examination Over – 4 0 diopters myopias can also be found
The method was rather unreliable for finding hyperopias presumably because no cycloplegic drops were used

Key words strabismus – hyperopia – myopia – anisometropia – fundus reflex – corneal reflex – photographic screening of vision defects and eye disorders in children

earlier article (Kaakinen 1979) the author presented a simple method for smus anisometropia or ametropia screening of children by photograph ith a conventional camera and flashlight the corneal and the fundus es *simultaneously The method and some experience of illustrative cases* presented in that first part of the investigation

establish the reliability of the method the results were compared with the test synoptophoric examinations and refraction measurements in the al strabismus and refractive error material in a double blind study The se of the investigation was to compare the sensitivity of the photography od with the clinical cover test in quite small angled strabismus cases too ı can be rather difficult to find especially in small children Straight eyed with great anisometropic or ametropic refractive errors which may cause opias or need refractive correction were also considered as possibilities imitations

Material and Methods

otal material was 128 patients 22 of them were strabismus patients of the nki University Eye Clinic The remaining 106 patients were cases with eye tions sent by the school health sister for ophthalmological examination The ı age of the patients was eight years because the latter group of patients pupils from the first and second grade of elementary school

ıe patients were first photographed from a distance of one metre as ıbed before (Kaakinen 1979) No mydriatic eyedrops were used before ography

ter photography a careful cover test in near fixation was performed The e of movement of the deviating eye in the cover test was expressed as ly observable or very small The latter indicated a just discernible move

ment mostly less than 5° Heterophorias were not mentioned because [illegible] naturally not to be found in the photographs After the cover test [illegible] 1% cyclopentolatehydrochloride (Cyclogyl®) was instilled in the eyes and was repeated after five min The objective refraction was then established [illegible] half an hour with a streak retinoscope The transparent ocular media [illegible] searched with a slitlamp biomicroscope and an ophthalmoscope by [illegible] brightness of the fundus reflex The fundus examination was performed [illegible] time with an ophthalmoscope The synoptophoric angles were measured [illegible] wards in the strabismus patients

An experienced strabismologist and a student nurse examined the p[illegible] graphs separately Neither knew the results of the clinical examination [illegible] patients They were first shown model photographs from the previous [illegible] taken by the same method (straight eyes very small eso and exotropias [illegible] and 5° and some larger eye deviations) They were asked to examine the p[illegible] graphs showing symmetrical and asymmetrical localisation of the corneal [illegible] flexes Special attention was to be paid to very small asymmetrical [illegible] the corneal reflexes if uncertain it could be denoted with ± (presumably [illegible] metrical = strabismus suspect) The colour or the lightness of the both [illegible] reflexes or the size and localisation of the possible light crescents in the [illegible] were to be noted as equal or asymmetrical If the fundus reflexes were [illegible] metrical the lighter fundus reflex should be marked Noticeable anisoc[illegible] should be marked

The results from the examinations of the photographs were compared [illegible] the clinical examinations of the patients In the case of the astigmatic [illegible] errors spherical equivalents were used

Results

All the patients had normal eyes in the fundus examinations The tra[illegible] ocular media were clear in every case on both microscopical and [illegible] moscopical examination

Strabismus (Table I)

Twenty two of the 128 patients had a strabismus with the cover test The [illegible] of the strabismus was ≤ 5 with a synoptophore in 12 cases in all of the [illegible] angle with the cover test was indicated as very small In addition [illegible] two cases with a very small angle in the cover test in which the synop[illegible] angles were + 7 and + 13 In one case showing very small right hyp[illegible]

ie cover test the synoptophoric measurement was impossible because the as only one year old

etrical corneal reflexes in the photographs led the experienced nologist to identify 20 of the 22 strabismus cases The student nurse the same result except that she observed four false strabismus sus one false positive strabismus in the photographs that were straight the strabismologist had no false strabismus suspects and thus all his *106* ical corneal reflex cases had straight eyes with the cover test Both ismologist and the student nurse found one + 2 esotropia and one −2 *as strabismus suspects* and student nurse also had one strabismus t case with + 4 esotropia These were the strabismus cases that were *ted from the photographs*

er could find the two 1 esotropia cases in the photographs by examin localisation of the corneal reflexes but one was detected anyway se of the asymmetrical fundus reflexes and another because of the oria that was visible in the photograph These changes were noted by the smologist and the student nurse The strabismologist spent an average of onds in examining each photograph and student nurse about two ds longer

smus and anisometropia (Table I)

trabismologist and student nurse obtained identical results in examining mmetry or asymmetry of the fundus reflexes Eighteen of the strabismus had asymmetrical fundus reflexes and in all of them the fundus reflex e deviating eye was lighter than that of the fixating eye Eleven of the ismus cases had 0–1 4 diopters anisometropia and eight of these had netrical fundus reflexes The anisometropia was over 1 4 diopters in 11 10 of them had asymmetrical fundus reflexes Hence four strabismus cases ymmetrical fundus reflexes and the angle of strabismus in all of them ery small (≤ 4) The pupils also were quite small in these cases

cases and anisometropia (Table II)

of 106 straight eyed cases 94 had anisometropia of between 0 and 1 4 ters 85 (90 %) of them had symmetrical and 9 (10 %) asymmetrical fundus Two of the nine asymmetrical cases had anisocoria and a darker reflex on the smaller pupil side In three cases the myopical crescents not quite equal in size which was the reason for the asymmetrical ap of the fundus reflexes The anisometropia in these three cases was 0.3 and one without anisometropia In two cases only were the fundus asymmetrical without anisometropia at all

Table I
Strabismus cases

case No	cover test	synoptophore	anisometropia	Photographic results			
				strabismologist		nurse student	
				corneal reflexes	fundus reflexes	corneal reflexes	fundus reflexes
1	very small left exotropia	− 3°	4.5	+	+	+	+
2	marked left esotropia	+ 14°	1.0	+	+	+	+
3	marked left exotropia	+ 6°	4.3	+	+	+	+
4	very small left esotropia	+ 2°	0.3	+	+	+	+
5	very small right hypotropia	± 0° vertical 4°	0.0	+	+	+	+
6	marked right esotropia	+ 11°	0.1	+	–	+	–
7	very small right [illegible]	[illegible] [illegible]tical 2°	0.0	+	+	+	+
[illegible]	[illegible]	+ 7°	1.8	+	+	+	+
	[illegible]	+ 17	[illegible]	+			

12	k 11 ft tr p a	+ 33	5	+	+		
13	very small right esotr pia	13°	0 0	+	+	+	+
14	very small left esotropia	+ 4°	1 9	+	+	+	+
15	marked left esotropia	+ 20	1 5	+	+	+	+
16	very small right hypotropia		0 0	+	–	+	–
17	very small left esotropia	+ 1	3 4	–	–	–	–
18	very small right esotropia	+ 5°	1 5	+	+	+	+
19	very small right exotropia	– 2	5 0	±	+	±	+
20	very small left esotropia	+ 2°	0 9	±	–	±	–
21	very small right esotropia	+ 5°	0 0	+	+	+	+
22	very small right esotropia	+ 4	4 3	+	+	±	+

+ asymmetrical
– symmetrical
± presumably asymmetrical
* a patient with anisocoria

Table II
Anisometropia (straight eyed cases)

Anisometropia	0–1 4	1.5–2.9	≥3 0
Asymmetrical fundus reflexes	9	2	5
Symmetrical fundus reflexes	85	5	0

The sensitivity and specifity of the method for anisometropia of 3 0 diopters
α = probability of false positive
β = probability of false negative

$$\text{sensitivity} = 1 - \beta = 1 - \frac{0}{5 + 0} = 1\ 00$$

$$\text{specifity} = 1 - \alpha = 1 - \frac{9 + 2}{(9 + 2) + (85 + 5)} = 0\ 89$$

The anisometropia was 1.5–2 9 diopters in seven cases of which two asymmetrical and five symmetrical fundus reflexes

The anisometropia was 3 0 diopters or more in five cases 3 0 3 1 3 1 and 8 9 diopters All of these had asymmetrical fundus reflexes. The probability that this result is dependent on a change is less than 0 001 (d f = 1)

Hyperopia (Table III)

(The deviating eyes are excluded from this material)

The hyperopia was under + 4 5 diopters in 95 eyes in none of them was light crescent seen in the fundus reflex

The refraction was between + 4 5 and + 6 0 diopters in eight eyes in three of which a light crescent appeared in the low part of the fundus reflex. The five eyes which did not have crescents in the fundus reflexes had smaller pupils than those with pupillary crescents

There were no refractions of over + 6 0 diopters among the straight eyes and deviating eyes were not included in this hyperopia material. But it may be mentioned that the only great hyperopias, about + 15 diopters, were in deviating eyes in each of which a marked hyperopic low part pupillary crescent appeared.

Table III
Hyperopia (straight eyed cases)

Refraction	< +4.5	≥ +4.5
Eyes with low part pupillary crescent	0	3
Eyes without low part pupillary crescent	95	5

a (Table IV)

deviating eyes are excluded also from this material)

refraction was less than −2.0 diopters myopia in 79 eyes of which only ad a small crescent in the upper part of the pupil The refraction in these yes was −2.0 comb cyl +0.5 ax 0 The pupillary crescent was absent in r myopias

myopia was from −2.0 to −2.9 diopters in 19 eyes of which two eyes n upper part pupillary crescent and 17 did not

e myopia was from −3.0 to −3.9 diopters in 16 eyes Nine of these had an r part pupillary crescent and seven did not

refraction was from −4.0 to −4.9 diopters in four eyes three had upper pupillary crescents and one in which the refraction was *−4.0 did not*

e myopia was *over* −4.0 diopters in *14* eyes (mean −5.5 diopters) all with r part pupillary crescents This result is statistically very significant 0.001 (χ test d.f = 1) The size of the crescents was roughly proportional e amount of the myopia but a very small difference in crescent size was in eyes with the same amount of myopia.

crescents of any kind appeared in association with refractions of less −1.75 diopters myopia or less than +4.5 diopters hyperopia and the ber of eyes within this range was 172

matism

e was over two diopters astigmatism in 11 eyes It was combined with erate hyperopia or myopia as direct astigmatism in all the cases It was not rnible from any changes in the photographs The same was true of the ler astigmatism in 90 eyes

anisometropia of 3 4 diopters which nevertheless had symmetrical [illegible] of the fundus reflexes This patient had an anisocoria that can [illegible] explained as the reason for the symmetrical fundus reflexes [illegible] of the deviating and more hyperopic eye would be lighter as [illegible] suitably shaded by the smaller pupil on the same side Very close [illegible] of the photograph afterwards showed some lightness beside the [illegible] in the low part of the fundus reflex of the deviating eye which [illegible] explanation This small change was not noted by either person [illegible] photographs

In hyperopia of + 4 5 to 6 0 diopters the light crescent appeared [illegible] part of the pupil only in three eyes and did not appear in five This [illegible] test as such unreliable for detecting hyperopia of this amount. [illegible] opias were only seen in the deviating eyes in the material and [illegible] hyperopic crecents appeared The patients were evidently accommodat[illegible] they fixed their gaze on the small signal light of the camera and the[illegible] hyperopic crescents did not appear in the quantities that might [illegible] expected in comparison with the results of the demonstration eye in the [illegible] article Further photographic examinations in cycloplegia should be [illegible] hyperopic cases in order to obtain more information on this point.

In myopia the crescent appeared in the upper part of the pupil [illegible] between − 1 75 and − 4 0 diopters and in over − 4 0 diopters myopia [illegible] eye (14 eyes average − 5.5 diopters) This result is statistically very [illegible] with chi square testing ($P < 0\ 001$ d f 1) Thus the method is [illegible] for screening high myopias without cycloplegia

The variable size of the pupils apart from accommodation is [illegible] reason for the variable appearance of the hyperopic and myopic crescents [illegible] the same refraction values Therefore photography in cycloplegia should be tested for more accurate screening of refractive errors.

Astigmatism did not cause any specific changes in the photographs Th[illegible] natural because the method measures the refraction roughly in the [illegible] meridian The horizontal meridian could also be measured by turn[illegible] source 90 which would require an extra photograph This would make [illegible] sible to estimate the presence of astigmatism from the differen[illegible] the fundus reflexes in the same eye photographed in the separ[illegible] according to the principle of static skiascopy Further experiments how[illegible] needed in this respect

The present study gives a favourable view of this type of [illegible] method for the objective screening of strabismus in children. [illegible] eyed anisometropias and myopias can also be found In screen[illegible]

plegic medication should be used This anyway often changes the natural smus situation of the patient

rthy of consideration is the fact that the manifest leukokorias and ies in transparent ocular media can also be discovered with the method se they cause noticeable changes in the fundus reflex photographs The raphs have standard scale Therefore follow upp measurement of the cor diameters and other visible eye and face dimensions are possible with the d without touching a child

Acknowledgment

re indebted to Associate Professor Seppo Sarna for the statistical calculations of udy

References

Bruckner R (1962) Exakte Strabismusdiagnostik bei 1/2–3 jahrigen Kindern mit em einfachen Verfaren dem Durchleuchtungtest *Ophtha mologica 144* 184–198

Bruckner R (1965) Praktische Übungen mit dem Durchleuchtungtest zur Fruh nose des Strabismus *Ophthalmologica 149* 497–503

lino S J von Noorden G K (1969) Heredity in microtropia *Arch Ophthal icago) 81* 753–757

ston E M von Noorden C K (1967) Microtropia a newly defined entity *Arch hthal (Chicago) 78* 272–281

nen K (1979) A simple method for screening of children with strabismus anisome pia or ametropia by simultaneous photography of the corneal and the fundus exes *Acta Ophthal (Kbh) 57* 161 171

ors addresses

Kaakinen, M D
katu 15 B 15
Kajaani 10
nd

ko Tommila Docent
rtment of Ophthalmology
ersity of Helsinki
nd

ACTA OPHTHALMOLOGICA VOL 57 1979

Department of Ophthalmology (Head Thore Lie Th mason Rikshospitalet The National Hospital University of Oslo

THE COLOUR RECEPTORS IN AMBLYOPIA INVESTIGATE BY SPECIFIC QUANTITATIVE PERIMETRY

BY

EGILL HANSEN

With a method combining static colour perimetry and the two-c threshold technique of Stiles characteristic sensitivities of the cone mechanisms have been studied in amblyopic and non amblyopic eyes.
For the red and green cone mechanisms a central depression of sensitivity was found in the amblyopic eye the depression was more pronounced the higher the degree of amblyopia For the blue cone mechanism no significant difference was found between the amblyopic and non amblyopic eye except at the fixation point where the mean threshold sensitivity was significantly higher in the *amblyopic* eye than in the leading eye.
By registration of blue receptor curves a distinct age factor was observed. In the younger patients (aged 10-16) the sensitivity level at peripheral positions for both the amblyopic and the non amblyopic eye was significantly higher than in the older patients (aged 39-50) This difference is evidently due to the higher absorption of blue light in the ocular media in older patients
In our cases with eccentric fixation the true fovea represents the relatively best sensitivity of the red as well as of the green cone mechanism.

Key words amblyopia – static colour perimetry – Stiles functions – age differences

Static perimetry has proved to be a suitable method for studying the [illegible] defects in amblyopia (Mackensen 1959 François et al 1968) By static perimetry under ordinary conditions no differentiation can be made between the red gr cone mechanisms (Sloan 1950) nor between the individual cone mech[illegible]

Received December 15 1978

ıration of different cone systems can be obtained by the method of e chromatic adaptation combined with static perimetry in the Gold perimeter (Hansen 1974) It is the purpose of the present work to report teristic findings in cases of amblyopia by use of this method

Material

e patients 10–59 years of age were examined The principle facts are in Table I There were 4 males and 8 females 5 had strabismic ambly nd 7 had anisometropic amblyopia Mild amblyopia (visual acuity better /?0) was found in 5 patients medium amblyopia (visual acuity 6/20) in and severe amblyopia (visual acuity 6/60 or less) in 6 patients All the ts had normal visual acuity (6/6) in their best eye except patient F in the leading eye had been lost by injury one year previously Four ts had eccentric fixation in their amblyopic eye and 8 had central n. Opthalmological examination was otherwise normal in all patients

Table I

Twelve amblyopic patients examined by specific quantitative perimetry

Age and sex	Type of amblyopia	Visual acuity	Fixation
39 M	Anisom	6/60	Central
49 M	Strab	2/60	Grossly periph Nasal
50 F	Strab	3/60	Parafov Nasal
16 F	Anisom	6/60	Central
?7 F	Anisom	6/10	Central
24 M	Anisom	5/60	Parafov Nasal
59 M	Anisom	6/12	Central
10 F	Strab	6/20	Central unstable
4? F	Anisom	6/12	Central
?6 F	Strab	< 1/60	Periph Nasal
11 F	Strab	6/1?	Central unstable
11 F	Anisom	6/?0	Central

Methods

The type of fixation was determined by means of a visuscope Col
was examined with the Ishihara s test (11th edition) and the AO-HRR
and in some patients with the Farnsworth s D-15 test Static perimetr
chromatic adaption was performed with a method which has been des
earlier (Hansen 1974 Hansen & Seim 1978) Narrow banded
filters (half band width 10–15 nm) were used As the targets were
eliptical their sizes were indicated as the angular diameter of a c
prising the same area The standard size of the target was 54 (object
The patients used correction glasses for the test distance during the ex
nation If correction was only necessary in the amblyopic eye neu
were used in the other eye The examinations were performed in a
order for the amblyopic and the non amblyopic eye

Results

Static perimetry curves for 2 patients obtained in the standard white ill
tion of the perimeter is shown in Fig 1 for one patient (F) the perimetric
obtained under scotopic conditions is also shown Patient D with a ce
fixation still had some asymmetry of his perimetric profile Patient F
parafoveal nasal fixation showed a displacement of the blind sport a
the peak sensitivity towards the nasal side By scotopic registratio
displacement though more irregular was seen

By central registration against blue and purple background the spe
sensitivity curves show the characteristic pattern of the red and green
mechanisms (Fig 2) The response curve of the amblyopic eye is m
reduced but otherwise identical with that of the non amblyopic eye

By registration of spectral sensitivity against a yellow background the
obtained in patient C were almost identical for the amblyopic and the
amblyopic eye the peak sensitivity being at about 439–451 nm wh
responds to that of the blue mechanism The registration here was at
position Object lights corresponding to the maximum sensitivity of the
cone mechanisms have been used for the perimetric registrations in the
lowing and are indicated in Figure 2 by vertical lines

Static perimetry performed in a blue background light is shown in F
There is marked difference between the normal and the amblyopic eye
patients with severe amblyopia The curves of the amblyopic eye are
depressed in the central part

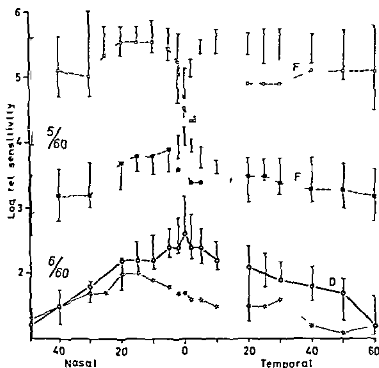


Fig 1

perimetry during dark adaptation (top curve) and in white standard illumination d/m²) performed in the only amblyopic eye of patient F and in both eyes of t D (lower curves) ?7 green target (λ = 512 nm) was used for registrations in ess and 15 and 54 white targets for the registrations shown by the middle and the curves respectively Dotted lines indicate the amblyopic eye for which the visual rates are ind cated The middle curve is displaced vertically by 1 log unit The bars indicate the normal variation (mean ∓ 2 SD)

ent F with an eccentric fixation has maximum sensitivity 2–5 to the side

ic perimetry curves obtained during adaptation to purple light are shown 4 There is a general depression of the sensitivity level of the amblyopic in the same way as was found for the red receptor mechanism However erences here are less pronounced Patient C with parafoveal nasal fix shows a maximum sensitivity of the amblyopic eye at 5 to the nasal in a manner similar to patient F

5 shows the perimetric profiles of 8 patients registered in yellow light a blue violet target There are some irregularities especially towards the

periphery the sensitivity level of the amblyopic eye being partly [illegible] partly below that of the leading eye Only patients F and J show [illegible] asymmetry expressing their eccentric fixation and in patient J a [illegible] was indicated near the fixation point Patient C apparently also had [illegible] fixation with his amblyopic eye during adaptation to the yellow light

A characteristic feature appears from the perimetric profiles the [illegible] sensitivity of the blue mechanism is not represented by the fovea, but [illegible] parafoveal region There is a distinct foveal dip in the perimetric [illegible] curve For 7 patients with a central fixation of both eyes (A C D H I L [illegible] the mean differences of threshold sensitivity found in the normal and [illegible] amblyopic eye at different perimetric angles are shown in table II [illegible]

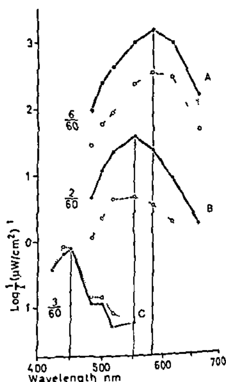


Fig [illegible]

Spectral threshold sensitivity registered against a blue background [illegible] lux) a purple background (B/Wratten 34 A [illegible]00 lux) and a yellow background [illegible] 589 nm 2000 lux) The registration is central (A B) and [illegible] nasal (C) The [illegible] patient A are displaced vertically by 2½ log unit and those of patient B by [illegible] Dotted lines indicate the amblyopic eye shaded area the normal variation [illegible] 1 SD) Angular size of target is [illegible]

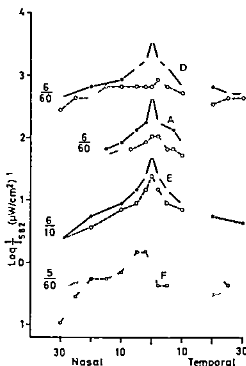


Fig 3

perimetry against a blue background (Wratten 47 165 lux) Target subtending gular size $\lambda_{m\,x} = 582$ nm Absolute threshold sensitivity is indicated correctly for ower graph those of the other patients are displaced vertically and separated by 1 Full line indicates the leading eye and stippled line the amblyopic eye The visual rates of the amblyopic eyes are indicated The shaded area indicates the normal variation (mean ± 1 SD)

l predominance of one eye over the other Only at the fixation point a significant difference whi h is in favour of the amblyopic eye being lly higher than in the non amblyopic eye ($P < 0\,01$)

ther characteristic feature is the relation of the age factor to the sensitivi vel of the blue mechanism The curves to the right in Figure 5 represent unger patients and are generally at a higher sensitivity level than the to the left representing the older patients In table III comprising 7 ents the mean threshold sensitivity of the older patients (39–50 years) at erent perimetric angles are compared with those of the younger patients 16 years) The values of the amblyopic and the non amblyopic eye were

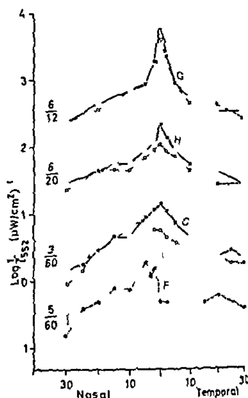


Fig 4

Static perimetry against a purple background (Wratten 34 A 900 lux) Target ... ing 54' angular size $\lambda_{max} = 552$ nm Explanation as for Fig 3

calculated together At 30° nasal position only inconstant response to the target was recorded in the older group while good sensitivity was indicated in the younger group Significantly higher mean values were found in the younger group at the perimetric angles 20° and 10° at the nasal side and 20° at the temporal side

Discussion

Contrary to what is expected determination of increment thresholds in the amblyopic eye was often more sharply indicated than in the non-amblyopic eye This is in accord with experience with partially sighted persons who are more often able to indicate their thresholds sharply when judging brightness contrasts than normally sighted persons (Lie 1974)

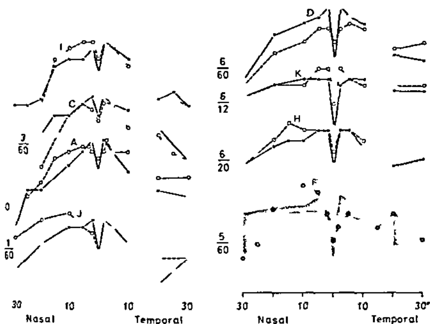


Fig 5

perimetry against a yellow background (λ = 589 nm 7000 lux) Target subtending 54 angular size λ_m = 451 nm Explanation as for Fig 3

Table II

a differences of threshold sensitivity for the normal and the amblyopic eye (S_N–S_A) n patients registered with a blue target against a yellow adapting background SD = *standard deviation*

Perimetric angle	S_N–S_A (Log units)	SD	Significance t	P
20°	0 19	0 29	1 72	
10	−0 01	0 19	0 18	
5°	−0 12	0 19	1 66	P > 0 1
2	0 02	0 23	0 28	
0	−0 21	0 15	3 76	P < 0 01
2°	0 04	0 20	0 49	
5°	0 03	0 05	1.55	
10	0 08	0 24	0 89	P > 0 1
20	0 02	0 21	0.26	
30°	−0 01	0 29	0 12	

Table III

Differences of threshold sensitivity in two groups of patien s tep end r 1 target against a yellow adapting background Sensitivity is calculated as log $\frac{1}{I}$ ($\mu W/cm$) [1] I = absolute irradiance from test spot so standard dev

	Peri metric angle	Mean sensitivity Group I (39–50 N = 3)	Group II (10–16 N = 4)	Differ ence	Common SD	Significance t	P
Nasal	30°	inconstant	0 40				
	20°	– 0 38	0 49	0 87	0 95	4 60	P<(
	10°	0 22	0 62	0 40	0 16	3 38	P<(
	5	0 35	0 69	0 34	0 19	2 39	00 < P<0
	2°	0 40	0 71	0 31	0 19	2 19	
	0°	0 08	0 26	0 18	0 34	0 0	P>(
Temporal	2°	0 43	0 70	0 27	0 17	2 11	005<P<0
	5°	0 35	0 63	0 28	0 23	1.59	P>(
	10°	0 26	0 58	0 32	0 28	1 47	
	20°	– 0 06	0 32	0 38	0 16	2 29	P<(
	30°	– 0 43	0 37	0 80	0 29	3 64	P<(

It is considered that the primary defect in amblyopic eyes lies in the ph vision mediated by cones in the central area For the red and green cone results obtained in our patients show that in both cone systems there is generalized depression of sensitivity in the amblyopic eye and this is more pronounced the higher the degree of amblyopia The same kind of general depression of sensitivity is found by ordinary static perimetry (Fra... 1968) and by registration of increment threshold spectral sensitivity (Zare... Szucs 1956 Harwerth and Levi 1977) On the other hand Wald and B... (1944) found the light thresholds of amblyopic eyes to be essentially n... (using 1–2 targets) By registration of the red as well as the green res... sponses (Fig 3 and 4) in eyes with eccentric fixation and severe ambl... there is a characteristic displacement of the peak sensitivity to respond the fovea The sensitivity at this point is only moderately reduced. On the contrary in patients with central fixation and severe amblyopia (A D ... is a considerable depression of the foveal sensitivity This is conn... conclusions by Mackensen (1959) using registrations in white light tha... foveal sensitivity in amblyopic eyes is relatively high when the fixat... eccentric while it is much depressed when the fixation is central

different response pattern was found for the blue receptor mechanism a significantly higher foveal sensitivity was found in the *amblyopic* eye n the leading eye This difference might be due to instability of fixation ie same difference of foveal sensitivity is found also in patients with a stable central fixation Evidence of greater receptive fields in amblyopic has been found by Flynn (1967) Lawwill et al (1973) and others The foveal sensitivity of the blue mechanism in amblyopic eyes may possibly cribed to differences in receptive fields implying also mechanisms of l inhibition A better threshold sensitivity to short wavelength stimuli to long wavelength stimuli in amblyopic eyes has also been reported by n and Szuchs (1956) and by Israel and Verriest (1972)

is interesting that patient C with an eccentric fixation apparently had al fixation after adaptation to the bright yellow light In cases where the fixation is not too fixed it is likely that a pure longwave illumination stimulate the foveal fixation It is therefore possible that the Na light of len th 589 nm is favourable for stimulating central fixation in cases of ble fixation Supporting this are the results obtained by Brinker and Katz 3) in patients with gross eccentric fixation where successful central fix was obtained after using red filters

age difference found in our patients by registration of the blue receptor onse may be explained by the higher absorption of the lens in the short range of the spectrum (Weale 1954) That the difference in sensitivity to targets is most pronounced in the periphery is consistent with a pure lens rption Yokoyama et al (1978) found differences in the response of the mechanism by ERG in phakic eyes according to age but in aphakic eyes onse of the blue cone system was almost the same regardless of age

ality of colour vision has often been found by a higher degree of yopia where it resembles that observed in normal eyes at eccentric positions s and Verriest 1967) Normal colour vision was found in all our ts by binocular examination Some discrepancy in the cases of severe lyopia may be ascribed to the reduced sense of form which may greatly ence the reading of pseudo isochromatic charts (Hansen 1963) Charac ically one patient (A) was not able to read the figures on the Ishihara s with his amblyopic eye though he could clearly indicate the colours By ic fixation Roth (1968) pointed out that colour deviation in amblyopic is the normal colour sense of the fixation area and that the colour sense fovea remains normal even when the fixation is extra foveolar Our ling of maximum sensitivity of the red and green mechanisms corresponding displacement of the fovea supports this

References

Brinker W R & Katz S L (1963) A new and practical treatment of ... ation Amer J Ophthal 55 1033–1035

Flynn J T (1967) Spatial summation in amblyopia Arch Ophthal (Ch ... 470–474

François J & Verriest G (1967) La discrimination chromatique dans l... strabique Docum opthal (Den Haag) 23 318–331

François J Verriest G & Verluyten P (1968) Comparison of the results of ... kinetic perimetry in the central region of the visual field of the amblyopic ... Arruga A ed Karger Basel International strabismus symp ... pp 45–50

Hansen E (1963) Factors causing uncertainty when conducting colour ... tests An Inst Barraquer 4 250–29?

Hansen E (1974) The colour receptors studied by increment threshold ... during chromatic adaptation in the Goldmann perimeter Acta oph... 490–500

Hansen E & Seim T (1978) Calibration of the Goldmann perimeter and ... used in specific quantitative perimetry Acta ophthal (Kbh) 56 ...

Harwerth R S & Levi D M (1977) Increment threshold spectral sensitivity ... metropic amblyopia Vision Res 17 585–590

Israel A & Verriest G (1972) Comparison in the central visual field of ... amblyopic eyes of the increment thresholds for lights of short and long ... Mod Probl Ophthal 11 76–81

Lawwill T Meur G & Howard C W (1973) Lateral inhibition in the central ... field of an amblyopic subject Amer J Ophthal 76 ...

Lie I (1974) Personal communication

Mackensen G (1959) Monoculare und binoculare statische Perimetrie zur ... der Hemmungsvorgange beim Schielen Albrecht v Graefes Arch Oph... 573–587

Roth A (1968) Le sens chromatique dans l amblyopie fonctionelle Docum ... (Den Haag) 24 113–200

Sloan L L (1950) The threshold gradients of the rods and the cones in the ... adapted and in the partially light adapted eye Amer J Ophthal 33 10 ...

Wald G & Burian H M (1944) The dissociation of form vision and light ... in the strabismic amblyopia Amer J Ophthal 27 950–963

Weale R A (1954) Light absorption by the lens of the human eye Op... 107–110

Yokoyama M Yoshida T & Uji Y (1978) Responses of the blue cone system in ... and aphakic eyes 15 ISCERG symposium Ghent 1977 Abstract in ... newsletter 33 1978

Zanen J & Szucs (1956) Les seuil achromatiques et chromatiques en vision ... dans l amblyopie strabique Bull Soc belge Ophtal 11? 193–?06

Author s address

Egill Hansen Department of Ophthalmology
Rikshospitalet The National Hospital Oslo 1 Norway

Department of Ophthalmology (Head A F Deutman)
Nijmegen The Netherland

CLINICAL ELECTRO OCULOGRAPHY

BY

A. PINCKERS

In clinical routine EOG we are dealing with a base line or lightinsensitive potential and a lightsensitive potential the latter consisting of at least two different oscillations a fast negative and a slow positive one Any judgement of EOG without referring to the absolute level of the baseline is an incomplete one It is no longer justified to refer to the Arden ratio as a unique parameter for EOG normalty or abnormalty Our knowledge about the generating mechanisms of the different EOG components is insufficient Clinical EOG examination is one way to better understanding Statistical evaluation is not easy because of the considerable inter and intraindividual variations The study of uniocular diseases or affections may solve some of our problems

Key words electro-oculogram – slow oscillation – fast oscillation – base line – Arden ratio – A-criterion

phthalmic literature the clinical electro-oculogram is usually expressed as a ient the lightpeak/darktrough ratio (Lp/Dt) of Arden The Lp/Dt ratio reflects aspect of the EOG the so-called slow oscillation or lightpeak The Arden ratio is used to classify a routine clinical EOG as normal subnormal or disturbed in EOG with a normal Lp/Dt ratio can be definitely abnormal because there is thologically lowered base line The purpose of this study was to evaluate routine cal EOG recordings at the Nijmegen Ophthalmic Clinic in order to get an ression of the possibilities of EOG examinations The results of this analysis are ussed in the present paper

ived November 2 1978

Materials and Methods

The material for this study consists of about 3000 patients examined [illegible] clinical conditions The EOG examination is the same as described in [illegible] studies (Pinckers & Thijssen 1969 1974 Thijssen et al 1974)

The Initial value (Iv) represents the first registration The Darktrough [illegible] mean of the measurements at the 6th 8th 10th and 12th min. The [illegible] is the mean of the 4 maximum values during light adaptation if no [illegible] present the Lp is the mean of the registrations between the 7th and 11th [illegible] the beginning of the light adaptation In every EOG we calculate the [illegible] ratio and the A-criterion A = Lp – (0 61 × Iv + 0 91 × Dt) where [illegible] intercept of the regression plane on the Lp axis for normal eyes [illegible] Thijssen 1969)

Normal values

The group of patients comprises also normal patients for example during [illegible] examinations As with the other patients these normal patients as a rule [illegible] familiar with the examination procedure This is a kind of continuous [illegible] the standardization of the technique and at the same time an ideal group [illegible] compared with the other patients There was no significant difference between [illegible] 80 normal eyes and the original 76 eyes who served for the standardization of [illegible] EOG technique (Pinckers & Thijssen 1969) I therefore decided to consider [illegible] normal patients as control group (Table I)

As published elsewhere we found no systematic differences between the right and left eye (Pinckers & Thijssen 1969) The results of right and left eyes therefore [illegible] be taken together in calculations There are however uniocular diseases [illegible] consequently calculated right left differences with regard to the Iv and the [illegible] The right left difference is expressed as the percentage of the highest value [illegible] example IvRE ≈ 550 µV IvLE = 520 µV Iv diff = $\frac{550-520}{550} \times 100\% = 5\%$

Table I
Normal EOG values (80 eyes 40 normal patients)

	Iv	Dt	Lp	Lp/Dt	[illegible]
mean (µV)	502	389	8 6	9 51	+10
stand diviation (µV)	176	143	29?	0 4?	[illegible]
mean – 2 SD (µV)	250	175	437	1.5?	[illegible]
lower 5% limit (µV)	250	193	450	1 80	[illegible]
lowest value (µV)	150	112	325	1 63	

Iv diff was 13 8% In 40% of the normal patients Iv diff < 10% in 10% was 10% and in 50% Iv diff was more than 10% The mean Lp/Dt diff was In 95% of the cases Lp/Dt diff was less than 20% in 87 5% less than 15% less than 10% and in 42 5% less than 5%

tive potential

tsensitive component of the EOG consists of at least two components a with a culmination time of approximately 9 min and a fast oscillation culmination time of 1 to 2 min (Kolder & North 1972)

atio and A-criterion

ow oscillation essentially represents the light rise or light peak (Lp) It is this ich is used in the form of the Lp/Dt ratio as the criterion for clinical EOG is no sharp delineation between a normal and a definitely abnormal Lp/Dt In order to obtain a better separation between normals and abnormals we uced the A-criterion in 1969 The A-criterion is calculated on the basis on alues (Iv Dt and Lp) the Arden ratio on two values (Dt and Lp) Since 1968 used both the Arden ratio and the A-criterion Table II gives the results of ion of 512 consecutive recordings From these I selected cases in which we pect a normal EOG for example in congenital functional anomalies albinism rriers of ocular albinism

a 95% limit the Arden ratio (91 4%) is better than the A-criterion (71 6%) ear that the Iv is responsible for this difference The Iv reflects the pre tion period In a darkadapted situation the corneoretinal standing potential

Table II

ratio and A-criterion in 512 consecutive EOG recordings Out of these 81 recordings on the basis of the clinical diagnosis were expected to be normal

	No	Lp/Dt ≥180	150 ≤ Lp/Dt <180	Lp Dt <150	A ≥ +83	o ≤ A A < +83	A < o
normal	81	74	7	0	58	23	0
norm (%)	100	91 4	8 6	0	71 6	18 4	0
in	512	223	91	198	172	103	237
(%)	100	43 6	17 8	38 7	33 6	20 1	46 3

Table II
Some examples of selective impairment of a single EOG mecha[illegible]

Base line involvement

Case No	diagnosis	eye	Iv	Dt	Lp	Lp Dt	[illegible] diff	[illegible]
693	normal	RE	420	200	900	4.50		[illegible]
	perf injury	LE	50	50	270	5.40	55	[illegible]
985	normal	RE	400	380	1000	2.63		
	perf injury	LE	80	80	400	5.00	80	[illegible]
4433	normal	RE	500	450	1200	2.66		
	perf injury	LE	200	200	500	2.50	60	[illegible]
5358	choroid effusion	RE	200	225	539	2.39	60	[illegible]
	normal	LE	500	413	989	2.39		

Lightpeak involvement

Case No	diagnosis	eye	Iv	Dt	Lp	Lp Dt	Lp Dt diff	[illegible]
692	siderosis	RE	450	429	797	1.90	41	<
	normal	LE	400	400	1356	3.39		
974	normal	RE	400	3[illegible]6	856	[illegible]43		
	macular disease	LE	350	387	581	1.50	54	<
1742	siderosis	RE	400	350	600	1.[illegible]	49	<
	normal	LE	450	400	1000	2.50		
1830	central a occl	RE	1000	800	1000	1.25	35	<
	normal	LE	1000	800	1550	1.94		

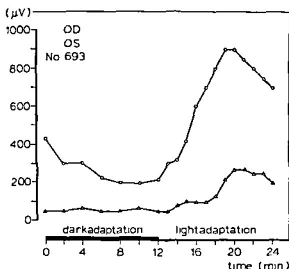


Fig 3

Impairment of the base line OS by perforating injury Normal Lp/Dt ratio of the left eye although the absolute value of the lightpeak is diminished
See also Table IV case No 693

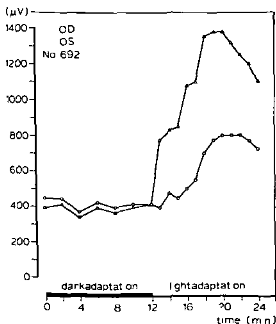


Fig 4

...tive impairment of the lightpeak OD in siderosis oculi The base line is not affected
See also Table IV case No 692

References

Alexandridis E (1976) Extraretinal influences in the EOG potential ...

Arden G B Barrada A & Kelsey J H (1962) New clinical test of retinal function based upon the standing potential of the eye Brit J phys Med 46 449

Arden G B & Kelsey J H (1962a) Changes produced by light in the standing potential of the human eye J Physiol (Lond) 161 189–204

Arden G B & Kelsey J H (1962b) Some observations on the relationship between the standing potential of the human eye and the bleaching and regeneration of ... J Physiol (Lond) 161 205–226

Francois J Verriest G & de Rouck A (1957) L'electro-oculographie en tant que ... fonctionnel de la retine Progr Ophtal 7 1–67

Francois J & de Rouck A (1973) Valeur comparative de l'electro-retinographie et de l'electro-oculographie Ann Oculist 206 235–243

Heilemann I & Bastian A (1968) EOC in cases of thrombosis of the vena centralis ... Proc VIth ISCERG Symposium Erfurt 83–87

Imaizumi K Takahashi F Yoshida C & Ogawa K (1968) The origin of the ... oculogram (EOG) Proc VIth ISCERG Symposium Erfurt 99–46

Kolder H (1967) Automatic recording and evaluating oscillations of indirectly measured corneo retinal potential in man Med res Engineering 5 ...

Kolder H & North A W (1967) Oscillations of the corneo-retinal potential ... Ophthalmologica 152 149–160

Pernice D & Taumer R (1976) EOG during iritis Bibl Ophthal 85 ...

Pinckers A & Thijssen J M (1969) The A-criterion in electro-oculography ... ISCERG Symposium Istanbul 284–288

Pinckers A & Thijssen J M (1974) Flat type electro-oculogram (EOG) ... 429–440

Pinckers A (1976) The lightsensitive negative component in clinical electro-... Docum ophthal proc series vol ... 345–341

Thijssen J M Pinckers A & Otto A J (1974) A multipurpose optical system for electrodiagnosis Opthalmologica 168 309–314

Szmigielski M (1968) EOG in comparison with ERG and psychophysical ... evaluation of retinal function Proc VIth ISCERG Symposium Erfurt ...

Author's address

Dr A Pinckers Department of Ophthalmology St Radboud Ziekenhuis Philips van Leydenlaan Nijmegen The Netherlands

Department of Ophthalmology (Head A Oksala)
Unn ersity Ce tral Ho pital Turku Fi land

EFFECT OF OILY VEHICLE ON OCULAR PILOCARPINE CONCENTRATION

BY

LOTTA SALMINEN

The effect of 1 4% polyvinyl alcohol (PVA) vehicle and of castor oil vehicle on ocular pilocarpine concentration was studied by radioactive method in the rabbit eye Statistically higher radioactivities were measured from the anterior surface structures of the eyes dropped with oily ehicle when compared to PVA vehicle at 120 min It is concluded that the conjunctiva and the cornea serve as a drug reservoir for the longlasting drug effect observed in the literature after oily pilocarpine drops

Key word rabbit eye – pilocarpine concentration – eh cle – polyvinyl alcohol (PVA) – castor oil

e *m ıı o* effect of pilocarpine on the pupil in an oily solution is of greater degree l duration than the effect of the same amount of pilocarpine given in methyl ulose (Borgmann & Wuster 1973) polyvinyl alcohol (PVA) (Saari et al 1978a) or leous (Smith et al 1978) vehicles Also oily drops of pilocarpine induce lower intraocular pressure and smaller maximum diurnal variation than PVA drops ne drug (Saari et al 1978b)

In the present study the greater ocular penetrability of pilocarpine in oily vehicle emonstrated when compared to the same amount of drug in 1 4% PVA vehicle

Material and Methods

elve adult albino rabbits weighing 2 5 to 3 kg were placed in individual animal ers where they remained during the experiment Twenty µl of a solution of led and unlabelled pilocarpine was instilled onto the upper part of the cornea

ed January 8 1979

During the instillation the upper lid was pulled slightly away from the gl[...] and 120 min after the instillation of the drug the rabbits six in each gro[...] killed by a rapid intravenous injection of pentobarbital sodium (Nembu[...] eyes were immediately enucleated gently rinsed in running water and f[...] liquid nitrogen The dissection of the frozen eyes and liquid scintilla[...] ocular samples were carried out as described previously (Salminen 19[...]

The tracer drug tritiated pilocarpine in alcoholic solution (4 1 Ci/mmol [...] mCi/ml) and obtained from the New England Nuclear Corporation was e[...] porated to dryness under vacuum The tracer material was dissolved in 1 4 % [...] with 2% pilocarpine hydrochloride and in castor oil vehicle with [...] pilocarpine The PVA solution contained 0 004% benzalkonium chloride whe[...] the castor oil solution contained no preservative The final solution ha[...] counting activity of 1 11 × 10⁴ disintegrations per min per microliter of solution

The results are expressed in terms of radioactivity that is the nonradioa[...] material and the bioactive tritiated pilocarpine are ignored in the calculations T[...] carrier substances were not chemically identical in the two vehicles and [...] radioactive material was not re-examined for its bioactivity during the st[...] However extensive metabolization of the labelled material during the stud[...] unlikely (Chrai & Robinson 1974 Lazare & Horlington 1975) Thus the amount [...] radioactivity can be considered as a measure of the effect of the two vehicles [...] pilocarpine penetration into the eye

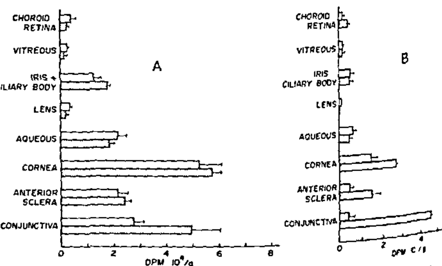


Fig. 1

Ocular radioactivity at 60 min (A) and at 120 min (B) after instillation of 10 µCi of [...] pilocarpine (4 1 Ci/mmol) in 20 µl of 1 4% polyvinyl alcohol (PVA) (open columns and [...] µl castor oil (shared columns) Carrier substance in PVA 2% pilocarpine hydroch[...] castor oil 2% pilocarpine 1 × SE

Results

oactivities obtained by liquid scintillation counting from various ocular struc are presented in Fig 1 At 60 min radioactivity in the conjunctiva was lly higher ($P < 0\ 01$) after castor oil vehicle when compared with 1 4% vehicle whereas at 120 min radioactivity in all anterior surface structures was lly higher ($P < 0\ 001$ or $P < 0\ 01$) in eyes with oily vehicle Low radioacti were measured in all parts of the inner coat of the eyeball

Discussion

results suggest a longer ocular surface contact time of drugs dissolved in oily icle and it is in agreement with the microscintigraphic study on petrolatum al oil ointment by Hardberger et al (1975) Because the enucleated eyes were d in water no information of the drug and vehicle in the conjunctical cul-de sac mixed with the tear fluid has been obtained

he longer surface contact time after oily vehicle led to a greater drug centation in the conjunctiva and cornea Drug in the conjunctiva and cornea iously served as a drug reservoir even after the wash-out of the drug and icle mixed with tear fluid and contributed to the longlasting pilocarpine effect r oily drops

he development of soluble drug vehicles for first-order kinetic systems is a great enge for the medical industry Optimal drug therapy of the eye could thus be ned with lesser drug induced side effects with smaller drug doses and with er applications

Acknowledgments

Ltd Pharmaceutical Manufacturers Tampere Finland kindly supplied the drugs and t animals

References

H & Wurster W (1973) Der einfluss unterschiedlicher Konzentrationen und ehikel auf die Pilocarpin Miosis II Unterschiedl che Vehikel *Klin Mbl Augenheilk 163* 1–56

S S & Robinson J R (1974) Corneal permeation of topical pilocarpine nitrate in the it *Amer J Ophthal* 77 735–739

Hardberger R Hanna C & Boyd C M (197) Effect of drug vehicles on o
Arch Ophthal (Chicago) 93 42–45
Lazare R & Horlington M (1975) Pilocarpine levels in the eyes of rabbits
application Exp Eye Res 21 281–287
Saari M Koskela P & Masar S E (1978a) Effect of vehicle on pilocarpine
Acta ophthal (Kbh) 56 496–503
Saari M Koskela P & Masar S E (1978b) Effect of vehicle on pilocarpine
hypotension Acta ophthal (Kbh) 56 489–495
Salminen L (1978) Cloxacillin distribution in the rabbit eye after intravenous
ophthal (Kbh) 56 135–143
Smith S A Smith S E & Lazare R (1978) An increased effect of pilocarpine on
application of the drug in oil Brit J Ophthal 62 313–317

Author s address

Docent Lotta Salminen
Turku University Central Hospital
Dept of Ophthalmology
SF 20520 Turku 52
Finland

Helmholt Institute of Ophthalmology
(Director E V Trutneva) Moscou U S S R

INFANTILE GLAUCOMA ELECTROPHYSIOLOGY

BY

A. V KHVATOVA, N A KOVALCHUK
and A I BOGOSLOVSKY

Thirty-eight children with congenital infantile glaucoma (hydrophthalmia) were examined using electrophysiological methods In contrast with adult glaucoma hydrophthalmia is characterized by early changes in the electroretinogram and rise of the threshold of electrical phosphene which points to serious pathological changes in the retinal optic nerve In such cases surgical intervention is indicated

Key words infantile glaucoma – electroretinogram – electrical phosphene

state of visual functions in children with congenital glaucoma (hydro-
almia) has been comparatively poorly studied partly because of the difficulties
ed by functional examination of children In particular little information is
ɔle on objective clinicophysiological examination (electroretinogram ERG
ɔ-oculogram EOG and electroencephalogram EEG) Francois et al (1956)
ned 91 patients with congenital glaucoma and noted in most of them changes
ual acuity field and adaptation These changes however were not specific to
ithalmia but could also be observed in other forms of glaucoma On the
r hand some other changes were discovered in the electroretinogram of nearly
patients (decrease in the amplitude of b-wave and other) not characteristic
pen angle glaucoma of adults Some increase was noted in the basal values of
EOG but the EEG remained unchanged Changes in the ERG of patients with
tal glaucoma were also observed by Pagani (1959) (cited after Jayle et al
)

racteristics of the phasic development of infantile glaucoma are usually based on

ived May 29 1978

clinicomorphological data and practically do not take into account the [illegible] functional state (Lagrange 1925 Cherkasov 1965 Kovalevsky 19[illegible]

In the present investigation it was attempted to establish a relationship between the stages of the periodization derived from clinicomorphological data [illegible] functional parameters in particular with the ERG and electrical sen[illegible] eye

Methods

Ocular examination included examination of the anterior segment of the eye [illegible] (oblique) illumination measurement of cornea diameter with a keratometer [illegible] biomicroscopy tonometry tonography

Small children were examined (including measurement of intraocular pres[illegible] anaesthesia (deep premedication) Visual acuity in children above 3 years was determ[illegible] current methods Determination of visual acuity in younger children were [illegible] children above 5–6 years the field of vision was examined on a projection perime[illegible]

The ERG was recorded on a commercial electroencephalograph with a pen recorder [illegible] New methods of functional diagnostics and ophthalmology Moscow 1[illegible] diagnostic significance of electrophysiological indices in the major diseases of the [illegible] neural apparatus Moscow 1976) For the leading off of biopotential silver electrode[illegible] used mounted into a contant lens They were fitted in each individual case from [illegible] devised for the examination of children The flash energy of the light stimulus [illegible] joules duration 50μsec visual angle of the stimulus ~9[illegible] distance from the patient [illegible] Both single and rhythmic stimuli were presented Very young and non-cooperative [illegible] were examined under conditions of profound premedication (nembutal [illegible] diphenylhydramine hydrochloride chloral hydrate) in doses appropriate to the [illegible] patient (Kovalchuk 1971a b) Examination began after a 40-min sleep period [illegible] laboratory illumination After the placement of electrodes the children remained in com[illegible] darkness for 6 min No mydriatics were used The children were examined in a [illegible] position in a light and electrical screened cabin All the electroretinogra[illegible] were performed on a natural pupil Patients undergoing miotic therapy [illegible] the evening before and the day of the examination and their pupil [illegible] noticeably from that of glaucomatous and healthy eyes

In older children (from 7 years and above) electrical sensitivity of the eye [illegible] investigated i e we were able to determine the threshold of electrical phosphene [illegible] technique developed at the laboratory of physiological optics (Bogoslovsky 1941 [illegible] the aid of the electrostimulator "TEST 1" designed by the experimental w[illegible] USSR Academy of Medical Sciences The threshold of electrical phosphene was deter[illegible] by the method of constant stimuli from minimum current intensity which p[illegible] the eyeball evokes a luminous sensation (electrical phosphene) [illegible] was also examined it is characterized by the highest frequency of intermittent [illegible] treble threshold intensity at which the sensation of flickering disappears The [illegible] electrical phosphene is a response to the electrical stimulus of the neurons [illegible] nuclear layer and fibres of the optic nerve (Bogoslovsky 1941 Potts 1969 and others [illegible] is a response to the stimulus of neutrons of the axial bundle of the optic nerve [illegible] 1963) The threshold current does not excite the photorecept[illegible] breakdown the visual purple

e control group consisted of 50 healthy children aged from 1 to 14 years examined in ection with a genetic consultation of families handicaped by hereditary eye diseases itis pigmentosa etc) As the amplitude of the a and b-waves does not change noticeably the first 2 years of life it was possible to compare the averaged data obtained for the e group with the parameters of the ERG of patients with different stages of congenital coma

Results

-eight children (67 eyes) with congenital glaucoma (hydrophthalmia) aged 1 to 14 years were examined using the methods described above According to markedness of pathological symptoms five stages of hydrophthalmia can be gnized No definite relationship between age and stage of the disease was d What is more in one and the same child the stage of the glaucoma was often erent in the right and left eye In most cases (21 children) an already developed ucoma was *diagnosed at the age of 3 to 7 years Hydrophthalmia was present in* initial stages in 11 eyes developed hydrophthalmia in 25 eyes advanced in 14 s nearly absolute in 11 eyes and absolute in 6 eyes

The early stages of the disease included the following findings an enlarged all, with a corneal diameter not exceeding 12 mm no dilated limbal vessels an nor chamber deeper than normal single ruptures of Descemet s membrane ucomatous excavation of the optic disc either absent or in the initial stage nioscopically ascertained embryonal tissue in the angle of the anterior chamber reased intraocular pressure In the developed stage the eyeball was markedly arged corneal diameter 13–14 mm limbal dilated ruptures of Descemet s mbrane confined corneal opacities deep anterior chamber hypoplasia of the embryonal tissue in the angle of the anterior chamber in many patients rked glaucomatous excavation of the optic disc and a high intraocular pressure

The symptoms of advanced hydrophthalmia were greatly enlarge eyeball l diameter above 14 mm strongly dilated limbal vessels multiple ruptures of Descemet s membrane corneal opacity anterior chamber very deep embryonal ue in its angle high intraocular pressure Visual acuity considerably reduced

n nearly absolute glaucoma photoperception was preserved with uncertain or rrect light projection

n children with absolute glaucoma vision was 0

Intraocular pressure was up to 27 0 mmHg in 12 eyes 28–32 in 21 eyes and 33 or more in 34 eyes

Visual acuity could be measured in 29 children (54 eyes) It was 0 3–0 4 in three 1–0 2 in eight and 0 01–0 09 in 27 eyes Photoperception with correct light ection was preserved in 10 eyes 6 eyes were blind In 9 small children (13 eyes) sual acuity could be not approximately determined

Table I

Mean parameters of ERG and electrical excitability of visual system in 38 [illegible] with hydrophthalmia

FRC (Empl.)		Reproduction of flickering		Electrical [illegible]	
a wave μV	b-wave μV	White light Hz	Red light Hz	Threshold of electrical sensitivity μV	[illegible]
25 ± 5.3	130 ± 19.1	36 ± 4.5	24 ± 4.[illegible]	1[illegible]8 ± 96.8	[illegible]

The basic parameters of the ERG, electrical sensitivity and lability in [illegible] hydrophthalmia are presented in Table I.

A distinct decrease may be noted in the values of the basic components of the ERG and electrical excitability. The change in these values with the progress of [illegible] disease are presented in Table II.

Discussion

The results obtained revealed a substantial difference between the functional [illegible] of the retina in children with hydrophthalmia as compared with that of [illegible] patients with primary glaucoma. The FRC of adults, even with absolute glaucoma [illegible]

Table II

FRG at different stages of hydrophthalmia parameters

Stage	Indices of the electroretinogram			
	a wave μV	b-wave μV	Rhythm reproduction: White light Hz	Rhythm reproduction: Red light Hz
Initial	40 ± 5.5	200 ± 14.8	49 ± 1[illegible]	[illegible]
Developed	32 ± 1.9	160 ± 8.47	43 ± 1.48	[illegible]
Advanced	16 ± 2.1	1[illegible]5 ± 9.6	30 ± 1.9	[illegible]
Nearly absolute	10 ± 1.8	70 ± 9.5	[illegible]1 ± [illegible]	0 [illegible]
Absolute	0 = abs	0 = abs	0 = abs	
Control group (healthy)	54 ± [illegible]5	270 ± 9.4	5[illegible] ± 4.7	[illegible]

a rule near to normal in respect to amplitude and frequency (Semenovskaya 959 Aladjoff et al 1959)
iled to discover a connection between the parameters of the ERG electrical vity and lability on the one hand and the level of intraocular pressure on the This absence of relationship is probably accounted for by the fact that in phthalmia the degree of functional disturbance of the retina is determined by ige of the pathological process rather than by the level of intraocular pressure moment of examination It may be assumed that the progressing enlarge of the eyeball with ensuring distension and thinning of the retina are nsible for the disturbance of visual functions This is confirmed by the data ed for patients with juvenile and secondary glaucoma in infantile glaucoma companied by eyeball enlargement the parameters of the ERG electrical vity and lability were far less affected by the progression of the pathological (Khvatova et al 1975) Thus in juvenile glaucoma normal amplitude and ncy parameters were observed occasionally (a wave 50–70 µV b-wave 0 µV rhythm reproduction in white light 50 Hz in red light 44 Hz) when yeball was not enlarged Conversely very low amplitude and frequency values always obtained with a greatly enlarged eyeball Thus the a wave was absent in of six eyes out of the fourteen eyes examined in one case it was 30 µV and nce its value reached 100 µV The b-wave was absent in the ERG of 5 patients al to 30–65 µV in 3 patients and 230 µV in one patient A corresponding was observed in the values of rhythm reproduction in white and red light patients with secondary glaucoma lingering high intraocular pressure without nlarged eyeball the amplitude and frequency parameters of the ERG and sensitivity were either normal or only slightly reduced a wave 52 µV ve 207 µV rhythm reproduction in white light 44 1 Hz in red light 33 6 Hz hold of electrical sensitivity 56 µA lability 40 4 Hz

Conclusions

he amplitude and frequency parameters of ERG electrical sensitivity and ty are lowered in patients with hydrophthalmia

The degree of decrease in these parameters rises with the progress of the ological process from the initial to the absolute stage of congenital glaucoma ng the initial stage the electrophysiological parameters are as a rule normal s with the transition from the initial to the developed and advanced stage he absolute stage no ERG is elicited electrical sensitivity and lability are greatly iced (not infrequently no electrical phosphene is evoked)

The character of the ERG electrical sensitivity and lability in hydrophthalmia s to damage of both the inner and outer layers of the retina apparently

resulting from important structural changes produced in the … eyeball by high ocular tension

4 Normal parameters of ERG electrical sensitivity and lability … stabilized process when conservative treatment is possible Changes in … reduction of electrical excitability of the visual system are signs of a … pathological process requiring early surgical intervention

References

Aladjoff S C (1960) Contribution a l'etude de la glaucoma precoce … retinographique *Electroretinographia Brno* 235–242

Bogoslovsky A I (1944) Problems of the electrical sensitivity of the eye … *kor ptike* V 2 136–172

Bogoslovsky A I (1969) The electroretinogram and its clinical importance … *mology* 5 69–75

Bogoslovsky A I (1973) *New method of functional diagn … ophthalmol* …

Chkerkasov I S (1965) *On the diagnostics of congenital glaucoma* In … children and other problems of children ophthalmology pp 101–108 Kiev

Eroshevsky T I & Tokareva B A (1971) *Congenital infantile glaucoma* … Moscow

Francois J Verriest G & De Rouck A (1956) Les fonctions visuelles dans le … congenital *Bull Soc belge Ophtal* 114 536–556

Jayle C E Boyer K L & Saracco I B (1965) *Effect of …* Paris

Katznelson A B (1960) Congenital infantile and juvenile glaucoma In … *diseases* Moscow

Khvatova A V (1971) On the diagnostics and treatment of main forms of … pathology of the vision organ in children In *Materials of the … problems of prophylactics pathogenesis and treatment of … of the eye in children* … Moscow

Khvatova A V Kovalchuk N A & Bogoslovsky A I (1972) A comparative … characteristic of congenital infantile glaucoma *Vestnik Ofalmol* no 3 90–95

Kovalevsky E I (1969) New classifications of cataracts glaucomas and … children In *Age-dependent peculiarities of the eye of children in norm and in …* Moscow

Kovalchuk N A (1971) Electroretinographic investigation of children in a … premedication *Vestnik Oftalmol* no 5 60–63

Kovalchuk N A (1971b) Electroretinographic investigation of children In … *and optic nerve* Moscow

Lagrange F (1925) Traitement du glaucome infantile *Arch Ophtal* (P…) …

Pagani J (1959) Contributo a l esame funzionale de l occhio idroftalmico … 112–128

Potts A M Inoue J & Buffum D (1969) The electrically evoked response of the … system (EER) *Invest Ophthal* 7 No 3 269–278

Semenovskaya E N (1963) *Electrophysiological investigation in Ophth…*

Authors' address Moscow Institute of Opthalmology Sadovaja Chernogrjaz… 14/19 Moscow U S S R

From the University Eye Clinics of Odense (Head P M Møller)
and of Rigshospitalet Copenhagen (Head F Creg rsen)
and Eye Pathol gy Institute (Head S Ry Andersen) University of Copenhagen Denmark

ORBITAL ABSCESS IN TWO NEONATES DERIVING FROM CONJUNCTIVAL MALFORMATIONS

BY

DANUTA MARUSZCZAK JENS C KRARUP
and HANS C FLEDELIUS

A report is given of two infants who developed an orbital abscess within their first month of life Generally they were unaffected and malignancies were initially suspected Ultrasonography showed partly cystic partly solid lesions The likely infectious foci were conjunctival malformations a mucosal fistula in case No 1 a conjunctival cyst in case No 2
There was rapid and complete recovery after surgery The infections were caused by Staph aureus and by diplococcus pneumoniae respectively
Additional findings Infant No 1 had a fibroma of the tongue Infant No 2 had pes equino-varus

Key word orbital abscess – orbital tumours – orbital ultrasonography – conjunctival malformations

orbital infections occur only sporadically in Denmark The present paper is with two cases affecting *neonates* where history and clinical findings initially to a *suspicion of orbital malignancy* The two cases are exceptional in that *ival malformations* appeared as the likely focus of the infections

1

-days-old male infant number two of two was admitted to Odense Hospital because of ess and swelling of the tissues of the left orbit

ly history Congenital malformations and ophthalmic disorders were not known In ticular manifestations of von Recklinghausens disease could not be disclosed

ived January 19 1979



Fig 1

Case No 1 A) prior to surgery proptosis of the left eye with redness and ... and a 5 mm lid lag B) three days after surgery C) one month after surgery ... the region The arrows (A and C) indicate the rounded tongue ...

Gestation and delivery were uneventful birth weight 3900 g General ph... was normal except for a round dense tumour measuring 10 by 10 mm (Fig 1 A C ... apex of the tongue

Eyes Two weeks old he had suffered from a slight conjunctivitis of the left eye ... only a few days and treated topically with chloramphenicol

Three days prior to admission proptosis and redness of the left eye ... with swelling of the ipsilateral eye lids Chloramphenicol eyedrops and ... were given immediately apparently without any effect.

On admission there was a 4–5 mm left lid lag and eye motility was ... directions The cornea was clear only slight chemosis moderate swelling of the ... particular the lower one increased retrobulbar resistance The fundus appea... slight stasis The right eye and orbit appeared entirely normal Repeated c... left conjunctival sac were negative

Generally the child was unaffected. Rectal temperature was 37 ... count normal but the ESR was high 70 mm h Except for the ... abnormalities were found at oto-rhino-laryngological examination X ray ... orbits without bone destructions Maxillary and ethmoidal sinuses were ... signs of sinusitis

After two days of systemic treatment with meticillin + streptomycin and ... topically without clinical improvement the infant was transferred ... suspicion of a malignant tumour of the orbit (Fig 1 A)

CT scan was inconclusive (technical difficulties due to unrest and the ... infant) but *ultrasound examination* revealed a 20 × 30 mm lesion in the ... the diseased orbit on the nasal side of the eye and also behind it (F... was predominantly cystic Low reflecting structures were seen in the ... ultrasound abscess or sarcoma were the most probable diagnoses.

During "compression test" with the transducer (the cyst proved to be ... compressible than the eye) a whitish discharge came out in the ... fornix through a fistula with smooth mucosal walls There was no ... relation to probing of the fistula

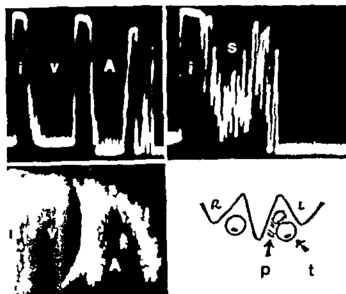


Fig 2

Ultrasonographs in case No 2 *Transbulbar* examination (marked by t on the schematic drawing) shows the eye (i = initial echo v = sound homogeneous vitreous space) *before* the predominantly cystic retrobulbar tumour by A-scan (top-left with low reflecting structures = abscess cavity) and B-scan (bottom left) Medial parabulbar (p) A-scan shows a solid lesion (S) anteriorly in the orbit (top-right)

...cal exploration was then performed through the lower lid (cf Fig 1 B) in a plane ...o the orbital floor At a depth of about 15 mm an abscess cavity was found *Cultures* ...d Staph *aureus*

...r surgery there was complete recovery and there has been no recurrence Both eyes ...pear entirely normal (Fig 1 C) with normal motility and ophthalmoscopy

...lementary examinations Chromosome pattern normal Urine excretion of glucose ...mino acids also normal

...o 2

...l female infant number two of two was admitted to Rigshospitalet because of a growing tumour of the left orbit.

...ily history negative

...m n and delivery uneventful Birth weight 3350 g General physical examination was ...ept for slight pes equino-varus

...en days after delivery a tumour becdame visible in the left lower lid Two weeks later it ...ribed as a 10 × 10 × 10 mm hard nodule covered by unaffected skin

...ssion it had grown to 20 × 15 × 18 mm (Fig 3) It was still dense but reddening ...skin and small cutaneous phlebectasiae had now developed and the eye was displaced ... Fundus appearance was normal in both e es and the right orbit was normal



Fig 3

Case No 2 with displacement of the left eye due to a 'tumour of the left lower lid'. Transbulbar ultrasonography (t on schematic drawing) shows a sequestrated abscess (A = abscess) before the eye (v = vitreous space) by A scan (top-left) and B (bottom left) On parabulbar examination (p) the abscess cavity is detected (A scan top right B scan bottom right)



Fig 4

(Eye Path No 186/78 a) Histological section of the conjunctival cyst in case 3 L = lumen Goblet cells staining dark with P A S (arrows) are seen in the nonkeratinized epithelium (× 5)

ly the child was unaffected Haemoglobin normal ESR 8 mm/h white blood cell 19.2 mia 1 8) " being lymphocytes 15 neutrofilic granulocytes

There was no destruction of the facial bones CT scan showed a soft tissue tumour in -rior and inferior part of the left orbit

t graphy A cystic lesion however with a solid anterior part was found in the basal quadrant of the left orbit located anteriorly and without evidence of retrobulbar hanges (Fig 3)

iopsy was planned On incision of the left lower lid abundant pus appeared and a e lesion could be excised

revealed diplococcus pneumoniae No fungi

ophy (Eye Path Inst No 186/78a) showed a conjunctival cyst (Fig 4) lined by a well entiated multilayered non keratinizing epithelium with numerous goblet cells Inflam changes were seen in the adjacent stromal ti sue and in one corner of the specimen lacrimal gland tissue was noticed

-operative course was uneventful There were no subsequent recurrences

Discussion

al tumours in infancy and childhood present many problems concerning early iosis and efficient therapy Therefore centralization to a multi specialty team een advocated in earlier reports (Eldrup-Jørgensen & Fledelius 1975 Fledelius)

e efforts are reflected by the present report which deals with two neonates were transferred (case No 1) or directly admitted (case No 2) to Rigshospitalet nhagen due to suspicion of rapidly expanding tumours of the orbit This ic concept was based on the following features

Both infants were *generally unaffected* especially without fever or other signs of is Laboratory tests however revealed a markedly elevated ESR in case No 1 a slight leucocytosis in case No 2

The *usual neighbouring sources* of orbital infections (cf Burnard 1959 Esente & Buono 1960 Duke Elder & MacFaul 1974 Jazbi & Ritter 1977) were *not* sed In particular there were no roentgenological changes of facial or cranial the ethmoidal and maxillary sinuses were small and normal and the lacrimal unaffected

The lesions *presented as localized tumours* (Figs 1 and 3) There were no signs of orbital inflammation (cellulitis phlegmone) which otherwise is to be ed in *neonates* (Esente & Del Buono 1960 Kuranov 1976) whose defense offensive microorganisms is incompletely developed

Ultrasonograhy yielded concise information about the morphology of the presumed tumours showing cyst like lesions with however vividness [illegible] the pressure of the transducer which caused the purulent discharge through the fistula in the lower fornix during diagnostic manipulation [illegible] 1 (diagnostic ultrasound indeed!)

In both cases the diagnostic problem was finally solved through the [illegible] intended for biopsy specimens The offensive microorganisms were [illegible] aureus and pneumococcus respectively

Conjunctival malformation were the probable sources of infection [illegible] In case No 1 the conjunctival fistula appeared in combination with [illegible] malformation a fibroma of the tongue but there was no evidence of Recklinghausens disease malformations of the branchially derived [illegible] chromosome defects In case No 2 there was pes equino-varus beside the orbital cyst

According to the literature it is rather unusual for orbital infections [illegible] from conjunctival structures (Burnard 1959 Duke Elder & MacFaul 1974 [illegible] the two present cases

References

Burnard E D (1959) Proptosis as the first sign of orbital sepsis in the newborn [illegible] *Ophthal 43* 9–12

Duke Elder S & MacFaul P A (1974) The ocular adnexa part II [illegible] para-orbital disease In Duke Elder S (ed) *System of Ophthalmology* [illegible] Kimpton London

Eldrup Jørgensen P & Fledelius H (1975) Orbital tumours in infancy [illegible] 887–893

Fsente I & Del Buono C (1960) Esoftalmo acuto infiammatorio del lattante [illegible] 51–66

Fledelius H (1976) Embryonal sarcoma of the orbit *Acta ophthal* [illegible]

Jazbi B & Ritter F N (1977) Sinoscopy and sinus disease in children *[illegible] Amer 10* 71–80

Kuranov N I (1976) On peculiarities of orbitae complications of sinus [illegible] (Russian) *Zh ushn nos gorl n Bolezn 36* 13–16

Author's address

Danuta Maruszczak
Eye Dept F Odense Sygehus
DK 5000 Odense
Denmark

Unn ersity Eye Clinic
(Heads \ Dreyer J Edmund E Gregersen S Kessing H H Seedorff)
Rigsho pitalet Copenhagen Denmark and
the Dani h Inst tute of Biomedical Fngineering
(Head A \orthe ed) Glo trup Denma k

Short Communication

DIRECT OPHTHALMOSCOPY WITH SIMULTANEOUS COLOUR TELEVISION TRANSMISSION

BY

ERIK SCHERFIG JENS EDMUND and ALLAN NORTHEVED

The authors have developed a direct ophthalmoscope with simultaneous television transmission The ophthalmoscope is used clinically for student as well as postgraduate teaching and for intercollegial discussion of retinal changes which give rise to differential diagnostic difficulties

Key words ophthalmoscopy – television ophthalmoscopy

ect ophthalmoscopy with a hand held ophthalmoscope is carried out by one iner at a time and co-viewing is not possible

Therefore this method is difficult to teach because the instructor cannot be npletely sure that the student has observed the changes which have been pointed it to him

Television transmission has the advantage of a simultaneous reproduction of the ina and for many years it has been endeavoured to obtain this advantage Up till w the television cameras have been mounted on slit lamps and fundus cameras (a references is obtainable from the authors) but none of these methods is based n the reproduction of the daily clinical situation in which the retina is viewed 1 a hand held ophthalmoscope

On the basis of constant improvements in television technique through the past

at the 4 8th meeting of the Danish Opthalmological Society on 21st April 1979
ived May 18 1979

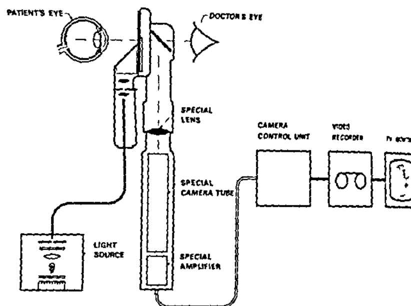


Fig 1
Schematic drawing of the television ophthalmoscope

years and in the attempt to create a clinical situation with a view to teaching intercollegial discussion we have developed a direct hand held ophthalmoscope with simultaneous colour transmission to a television screen and videorecorder

Technical data The apparatus consists of a light source a light transmitter a television ophthalmoscope a camera control unit a colour videorecorder a colour television monitor (Fig 1)

Light source and light transmitter Ordinary commercially available light source fibre light ophthalmoscopes

The television ophthalmoscope The light from the light transmitter is deflected reflected in the usual way through a lens and prism system to be focused on the retina As in conventional direct ophthalmoscopy the viewer sees the retina through a variable system of lenses which correct for the patient's and the examiner's refraction The light reflected from the retina is divided by a beam splitter partly to the examiner and partly to the television camera In front of the television camera a lens system is inserted permitting correction corresponding to the examiner's spherical refraction anomaly

e specifications for the television camera are

f camera Single tube colour camera 1 vith colour mesh	*V deo output* 1 volt sync negative PAL, in compliance with CCIR 75 ohms unbalanced
f camera 5 × 5 × 20 cm	*Signal t noise rat o* Better than 40 dB
m sy tem 5 linear 95 exposures/sec 1 interface orizontal frequency 15 695 kHz rtical frequency 50 Hz	*Len* 25 and 50 mm C mount
	Power requirement 230 V ± 10% 50 Hz
	Po er consumption 50 W
	Operating temperature 0–50°C
ard t rti n Less than 1%	*Colour temperatu e c mpen ation* 3200 K 4500 K 6000°K
etric di tort n Less than 2%	
h ti n capabil ty 250 lines	*Light distribution* 15% to the examiner 85% to the television camera
itivity 60 lux at diaphragm 2 5	

nera control unit colour television monitor and videorecorder The video signal is nsmitted in the usual way to the three above mentioned units and this affords an age on the television screen simultaneously with the ophthalmoscopy At the ne time the image is recorded on a video tape for later replay

The television ophthalmoscope is used in our Eye Department for simul neous viewing of retinal changes when such changes are to be demonstrated for ching purposes or when it is attempted to solve a differential diagnostic problem rough intercollegial discussion The use of videorecorders which can display lls increases the usefulness of the exposures

In particular the television ophthalmoscope is well suited for exposures of ildren bedridden patients or handicapped persons in whom it is difficult to otograph the fundus

Discussion

he image quality in the television transmission can be up to that of photographs it television images cannot be reproduced as paper prints or used for making nting blocks of the same quality as photographs of the fundus

Today television transmission is of such high quality with respect to resolution bility and colour recording that by virtue of its simultaneous reproduction it is ready being used instead of film for the demonstration of operations

Due to its small size and increased sensitivity the camera and ophthalmoscope developed by us introduce for the first time the televising of direct ophthal

moscopy. The quality is up to that which we have seen on television through a fundus camera, but our apparatus reproduces the ... in daily clinical practice in which the light moves over the central and peripheral parts of the retina.

Acknowledgments

The authors are grateful for grants from A. P. Møller og Hustru Chastine Mc-Kinney Møllers Fond til almene Formål, Fabrikant Einar Willumsens Mindelegat and ...

Authors address:
University Eye Clinic, Rigshospitalet, DK 2100 Copenhagen Ø, Denmark.

Departments of Ophthalm logy (Head Ulf Hallden)
and Medical Cent s (Head Lars B ckm n) Unr ersity of Umea Umea S eden

EIGHT CASES OF CONGENITAL ACHROMATOPSIA WITH AMBLYOPIA IN TWO PEDIGREES FROM NORTHERN SWEDEN

BY

WERNER POLLAND and STEFAN NORDSTRÖM

Two families from northern Sweden with a total of 8 patients with typ cal symptoms of congenital achromatopsia with amblyopia were studied In one of the families 4 affected children (3 brothers and 1 sister) also showed pallor of the optic discs and marked astigmatism The transmission of the disease was consistent with an autosomal recessive inheritance in both families The study confirmed that complete and incomplete achromatopsia might be different expressions of the same gene Six out of 13 near relati es of the achromatic patients showed minor colour vision defects suggesting a tendency towards heterozygotic manifestati on of the gene

Key wo ds achromatopsia – colour vision – autosomal recessi e inheritance – heterozygotic manifestation of recessive genes

eral types of so-called cone dysfunction syndromes are known among them genital achromatopsia with amblyopia (Deutman 1971)

atients with typical congenital achromatopsia show a complete lack of colour on and a visual acuity of about 0 1 bilaterally They also show nystagmus – cally in childhood – and pronounced photophobia. Colour matches can be over the entire range of the Nagel anomaloscope and brightness matches only end of the instrument (Fig 1) When tested with colour vision tests the romatic patients miss most plates in the pseudo-isochromatic series and make over the entire F M 100-Hue test with no definite axis

r ocular lesions associated with achromatopsia have been recorded e g lar changes pallor of the optic discs and astigmatism Waardenburg (1963) d others have considered pallor of the optic discs to be a familial symptom

January 22 1979

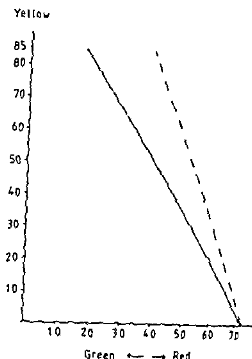


Fig 1

Positions on Nagel anomaloscope where brightness matches can be made by achromats (– – –) and incomplete achromats (——) (Krill)

although the significance of the pallor is unknown. According to [illegible] genuine atrophy of the optic nerve seems unlikely.

The hereditary character of the disease is obvious. Holm and Lo[illegible] presented a pedigree with 19 cases of congenital achromatopsia in a Danish [illegible] In Sweden Gothlin (1941) presented two pedigrees with a total of seven cases [illegible] also discussed the heredity from the point of view of earlier reports on the [illegible] Congenital achromatopsia is an unusual disorder. According to [illegible] prevalence is less than one in 300000. Cases have been presented in [illegible] American and Japanese literature since the 1890s; these are reviewed [illegible] Francois (1961) and Waardenburg (1963).

In most cases achromatopsia seems to be complete but incomplete forms [illegible] occur. In the incomplete forms the findings are not so pronounced as [illegible] complete forms and brightness matches in the Nagel anomaloscope are [illegible] in the green section (Fig 1). According to Franceschetti et al. [illegible] complete and incomplete achromatopsia may be different expressions of the [illegible] gene. They found the complete type in an elder and the incomplete [illegible] younger brother of the Danish family presented earlier by Holm & Lo[illegible]

naka (1957) suggested an X-chromosome linked transmission of the disease rding to Deutman (1971) incomplete achromatopsia can be X linked recessive possibly also autosomal recessive while complete achromatopsia always has an somal recessive mode of inheritance

Alford (1957a 1959) found deviation of red green colour vision and abnormal hing ranges in 3 parents of 2 patients with congenital achromatopsia when d with the anomaloscope indicating a tendency towards manifestation of the in a single dose

utman (1971) stressed the importance of more information on families with dysfunctions in order to establish the mode of inheritance and to delineate this lition more sharply from other similar conditions

aim of the present investigation was

to describe the clinical manifestation of the disease in two hitherto unpub-d families and

to study the mode of inheritance including manifestation of the gene in rozygotes

Materials and Methods

r out of 6 brothers and sisters in one family (Fig 2) and 3 out of 4 in another ily (Fig 3) were affected by the disease Both families lived in the same parish rotea) in the county of Vasterbotten in Northern Sweden Another patient (III Fig 2) first cousin of the patients in the first family was found in the course of study She and her family lived in Southern Sweden

he 8 patients were examined ophthalmologically as follows

Visual acuity and fundus examination

Colour vision testing with

a) BK and BII pseudoisochromatic plates
b) Ishihara plates (38 plates edition 1972)
c) American Optical Hardy Rand Rittler (HRR) plates
d) Farnsworth Munsell (F M) 100 Hue test
e) Anomaloscope (brightness matches)

he 6 parents and all the clinically normal brothers and sisters of the achromat ts were examined with the aim of detecting any possible heterozygotic festation of the gene One aunt of the second family and her son were also ned This examination was performed as described above with the following on

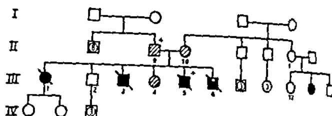
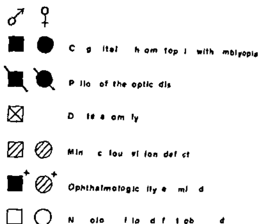
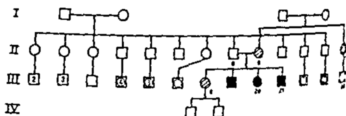


Fig 2
Pedigree of Family I





Fi 3
I edigree f Family II

2 e) Anomaloscope Red green deviation and matching range The tests were made with a Nagel anomaloscope calibrated according to Pickford (1[9] 5 1 The anomaloscope was calibrated by examination of 30 persons (1x we 10 men) with normal colour vision and without known near relatives with vision defects The limits at which the red green variable b led re be greener than the yellow standard was scale number 14 (only 9 per ns and (7 persons) respectively Most settings lay between scale number 14 and 4

‾ie brightness was always held equal by appropriate changes of the intensity ellow Matching range varied between two and four scale units
of the achromats (III 1 Fig 2) had 2 daughters who were clinically normal ere however too young to cooperate in a detailed colour vision examina

Results

affected patients in the same sibship of family I (Fig 2) had a visual acuity of ioderate astigmatism photophobia nystagmus and pallor of the optic discs first cousin in family I (III 14) had a visual acuity of 0 2 moderate iatism photophobia nystagmus but no pallor of the optic discs In family II

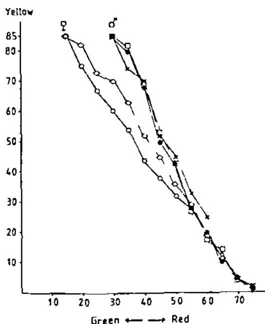


Fig 4

rightness matches in Nagel anomaloscope made by the fi e achromats in Family I (Fig 2)

◇ = III 1 (aged 31)
□ = III 3 (aged ‿9)
• = III 5 (aged ?0)
× = III 6 (aged 15)
O = III 14 (aged 13)

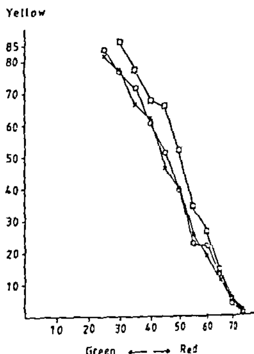


Fig 5

Brightness matches in Nagel anomaloscope made by the three achr[illegible]

O = III 19 (aged 2[illegible])
□ = III 20 (aged 1[illegible])
× = III 21 (aged 11)

(Fig 3) the 3 affected patients had the same typical symptoms of [illegible] visual acuity 0 1 photophobia nystagmus marked astigmatism [illegible] rule but no pallor of the optic discs When tested with pseudo[illegible] all the 8 patients missed most plates in all series All of them read plates [illegible] the Ishihara test correctly (The plates 14 and 15 are also the [illegible] darkadapted persons with normal colour vision when the [illegible] increased) Large errors were made over the entire F M 100-Hue [illegible] definite axis Anomaloscopically all could do brightness matches [illegible] section (Fig 4 and 5) There was a slight difference between the [illegible] family I (Fig 4)

The clinically normal relatives who were examined all had normal [illegible] and normal fundi Some had a slight myopia and hyperopia [illegible] significant astigmatism They all read BK and BII Ishihara and [illegible] When tested with F M 100-Hue test the 2 parents in family I (II [illegible] 2) and the aunt in family II (II 13 Fig 3) made significant errors [illegible]

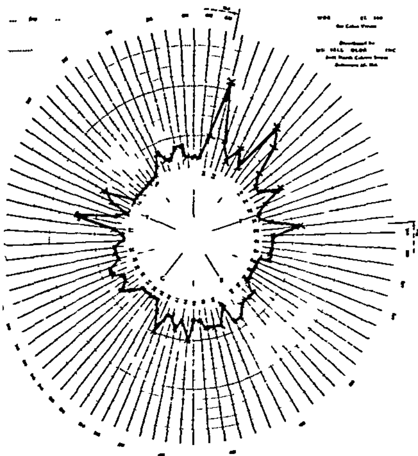


Fig 6
he Farnsworth Munsell 100-Hue error pattern of II 9 in Fam'ly I Error score 114

(Fig 6 7 and 8 Tables I and II) They had error scores which exceeded the 95 cent limit in the age group 50–59 given by Verriest (1963) and Krill & neiderman (1964) The other relatives had normal error scores

'hen tested with the anomaloscope (red green) the 2 parents (II 9 and 10) and sister (III 4) in family I (Fig 2) and the mother (II 9) the sister (III 18) and aunt (II 13) in family II (Fig 3) had minor defects (Tables I and II)

he transmission of achromatopsia in the pedigrees is consistent with an l recessive inheritance (see Fig 2 and 3)

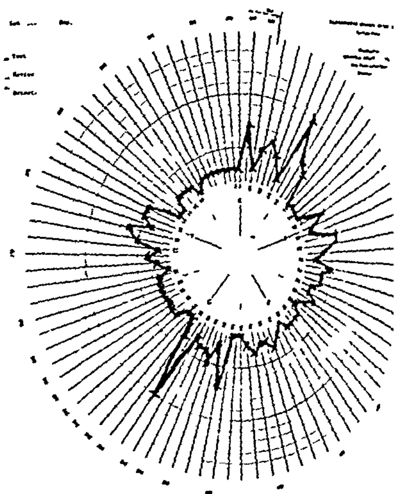


Fig [illegible]
The Farnsworth Munsell 100-Hue error pattern of II 10 in Family I [illegible]

Discussion

This study confirms that complete and incomplete achromatopsia [illegible] different expressions of the same autosomal recessive gene. The [illegible] achromatopsia in family I especially the first cousin (III 14 Fig 2) [illegible] a more incomplete form. She had better visual acuity and could [illegible] colour when seen on larger objects. We also think that this study [illegible] achromatopsia gene has a tendency towards heterozygotic man[illegible] near relatives had minor colour vision defects. Pickford (19[illegible]) found that [illegible]

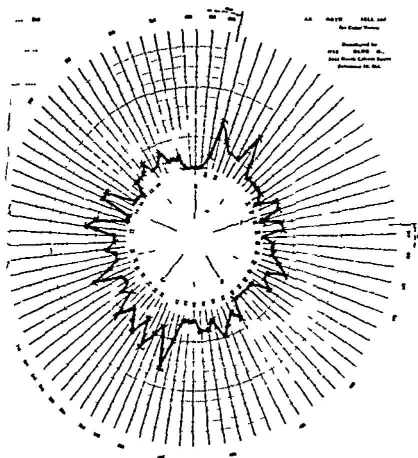


Fig 8
he Farnsworth Munsell 100-Hue error pattern of II 13 in Family II Error score 136

for red-green blindness had minor defects which accord in character with of major defect of their affected relatives In our study we found that a cousin (III 28 Fig 3) had a deuteranomaly His mother (II 13) and aunt) however showed minor defects with red deviation It is more likely that this festation of the gene for achromatopsia than of that for deuteranomaly F M 100-Hue test may sometimes be useful in detecting heterozygotes with a gene Tests on larger numbers of near relatives of patients with matopsia may confirm this

Table 1

Results of test on near relatives of achromats (Fam. [illegible])

Case	Anomaloscope test (red-green)	Blue
II 9 (aged 57)	Green deviation (scale number 36) Matching range abnormal (36–44) 9 scaleunits	Normal (Fig [illegible])
II 10 (aged 53)	Green deviation (scale number 35) Matching range abnormal (35–41) 7 scaleunits	Normal (Fig [illegible])
II 13 (aged 57)	Deviation not abnormal Matching range normal (40–43)	Normal
II 14 (aged 41)	Deviation not abnormal Matching range normal (39–40)	Normal
III 2 (aged 31)	Deviation not abnormal Matching range normal (41–42)	Normal
III 4 (aged 25)	Green deviation (scale number 36) Matching range abnormal (36–40) 5 scaleunits	Norm. (Farn [illegible] normal)
III 13 (aged 16)	Deviation not abnormal Matching range normal (39–42)	Normal
III 15 (aged 11)	Deviation not abnormal Matching range normal (41–42)	Normal

A genealogical study of the two families (Nordstrom & Polland [illegible]) [illegible] that the gene causing the disease has existed for at least 300 years [illegible] subpopulation in northern Sweden

Table II
Results of test on near relatives of achromats (Family II)

Case	Anomaloscope test (red green)	FM test
II 8 (aged 52)	Deviation not abnormal Matching range normal (38–41)	Normal
II 9 (aged 51)	Red deviation (scale number 48) Matching range abnormal (39–48) 10 scaleunits	Normal
II 13 (aged 54)	Red deviation (scale number 47) Matching range abnormal (38–47) 9 scaleunits	Abnormal (Fig 8)
III 18 (aged 23)	Red deviation (scale number 46) Matching range abnormal (38–46) 8 scaleunits	Normal
III 28 (aged 34)	Deuteranomaly	

References

ersen S R (1946) On congenital total colour blindness coexisting with heredo-labyrin ine deafness *A ta ophthal (Kbh) 24* 99–112

man A F (19 1) *The herid t ry dy trophies of the p terio pole of th eye* Koninklijke Van rcum & Co N V Assen

eshcetti A Jaeger W Klein D Ohrt V & Rickli H (1958) New facts resulting from a ysiological and genetic study of the large family of the island of Fur (Denmark) str Rep 18th Internat Congr Ophthal Sept Brussels *Exc Med 12 9 No 88* p CIII

is J (1961) *Heredity in ophthalmology* Mosby St Louis USA

iin F (1941) Die Erblichkeit Angeborener Totaler Farbenblindheit mit Lichtsheu *Acta (Kbh) 19* 209–218

E & Lodberg C V (1940) A family with total colour blindness *Acta Ophthal (Kbh) 18* 4–258

A E & Schneiderman A (1964) A hue discrimination defect in so-called normal carriers l ur vision defects *In e t Ophthal 3* 445–450

A E (1969) Congenital colour vision defects Symposium on surgical and medical agement of congenital anomalies of the eye *T an act on of the Ne Orl ans academy of h lm l gy* Mosby St Louis USA

str m S & Polland W (1978) A gene source for congenital achromatopsia (total colour ndness) in northern Sweden *Human Heredity* (To be published)

Pickford R W (1957a) Colour vision of achromat achromat parents ...

Pickford R W (1957b) A practical anomaloscope for testing ... colour blindness Brit J Physiol Optics 14 2–26

Pickford R W (1959) Some heterozygous manifestations of colour blindness ... Ophthalmol 16 83–95

Tanaka K (1957) Tests for partial sex linkage in total colour blindness ...

Waardenburg P J (1963) Genetics and Ophthalmology Vol II Blackwell Scientific Publ Ltd Oxford

Verriest G (1963) Further studies on aquired deficiency of colour discrimination ... Am 53 19...

Author address

Werner Polland M D Department of Ophthalmology
University of Umeå S 901 85 Umeå Sweden

Pickford R W (1957a) Colour vision of achromat achromat parents ...
Pickford R W (1957b) A practical anomaloscope for testing ... blindness *Brit J Physiol Optics 14* 2–26
Pickford R W (1959) Some heterozygous manifestations of colour ... *Ophthalm 1 16* 83–95
Tanaka K (1957) Tests for partial sex linkage in total colour blindness ...
Waardenburg P J (1963) *Genetics and Ophthalmology* ... *II* Black... Ltd Oxford
Verriest C (1963) Further studies on aquired deficiencies of colour ... *Am 53* 196

Author address

Werner Polland M D Department of Ophthalmology
University of Umeå S 901 85 Umeå Sweden

Department of Ophthu lmol gy (Head \ Ehlers)
Århus Kommuneho pital L nn ersity of Åa hus Denmark.

RACEMOSE HAEMANGIOMA OF THE RETINA

Report of three cases with long term follow up

BY

PETER BERNTH PETERSEN

Racemose haegiomas of the retina are rare developmental anomalies. The basic lesion is an abnormal arterio-venous communication.
Three new cases with long term follow up are presented. In two of the cases the first symptom was loss of vision due to retinal and vitreous haemorrhage In the third case gradual reduction of vision was due to vascular leakage into the macular area. The recent literature is reviewed and the association to midbrain haemangiomas discussed The ophthalmological differential diagnosis the prognosis and the present status in treatment is presented

Key ords racemose haemangiomas of retina – arterio-venous aneurysm of retina – developmental anomalies – intracranial arterio-venous aneurysm – retinal vascular decompensation

racemose haemangiomas of the retina are rare developmental anomalies Since gnus in 1874 described the condition less than 80 cases have been reported a of these from Denmark (Ehlers 1924 Frandsen 1950 Bech & Jensen 1958 ersen 1961)

he opthalmoscopic picture varies from a single well-defined anastomosis to a tumour like mass of arteries and veins Probably for this reason the ation has been described under a number of terms including racemose angioma, arterio-venous angioma arterio-venous aneurysm and cirsoid urism. The condition is not a true neoplasm as it has no potential for unlimited wth, production of metastases or infiltration nor does any characteristic stem l exist (Reese 1976) The term aneurysm is in fact also misleading as aneurysmal

ceived November 15 1978

Kelsey J H (1967) Variations in the normal electro-oculogram *Br J Ophthal*
Krogh E (1975) Normal values in clinical electro-oculography I ... dological investigations and distribution of the potential and time parameters *Acta Ophthal (Kbh)* 53 563–575
Krogh E (1976) Normal values in clinical electro-oculography II ... time parameters and their relation to other variables ...
Krogh E (1977) Normal values in clinical electro-oculography III ... the two dimensionless EOG parameters *Acta Ophthal (Kbh)* 55 ...

Author's address
Dr S A Jackson
Department of Medical Physics
Queen's Medical Centre and University Hospital
Nottingham Britain

Department of Ophthal ı logy (Head \ Ehlers)
Århus Kommunerho pıtal Unıversıty of Aa hus Denmark.

RACEMOSE HAEMANGIOMA OF THE RETINA

Report of three cases with long term follow up

BY

PETER BERNTH PETERSEN

Racemose haegiomas of the retına are rare developmental anomalies The basic lesion ıs an abnormal arterio-venous communication
Three new cases with long term follow up are presented In two of the cases the first symptom was loss of vision due to retinal and vitreous haemorrhage In the third case gradual reduction of vision was due to vascular leakage into the macular area The recent literature is reviewed and the association to midbrain haemangiomas discussed The ophthalmol gical differential diag nosis the prognosis and the present status in treatment is presented

Key words racemose haemangiomas of retina – arterio-venous aneurysm of retina – developmental anomalies – intracranial arterio-venous aneurysm – retinal vascular decompensation

racemose haemangiomas of the retina are rare developmental anomalies Since]nus ın 1874 described the condition less than 80 cases have been reported a of these from Denmark (Ehlers 1924 Frandsen 1950 Bech & Jensen 1958 gersen 1961)

he opthalmoscopic picture varies from a single well-defined anastomosis to a tesque tumour like mass of arteries and veins Probably for this reason the dition has been described under a number of terms including racemose mangioma arterio-venous angioma arterio venous aneurysm and cirsoid urysm. The condition is not a true neoplasm as it has no potential for unlimited with production of metastases or infiltration nor does any characteristic stem l exist (Reese 1976) The term aneurysm is in fact also misleading as aneurysmal

ed November 15 1978

dilatations are not the primary lesion although they occur secondary to large arterio-venous communications

The essential defect is one or more abnormal communications between retinal arterial and venous circulation probably due to an error in the anterior plexus of primordial cerebral blood vessels (R ... & ... Archer et al 1973 Reese 1976 Speiser 1978)

In two recent papers the term arterio-venous communications ... have been suggested for this condition (Baurmann et al 19.. ...)

A report of three new cases of racemose haemangioma is given below



Fig 1
Central part of fundus of the left eye in case No 1
A group 3 racemose haemangioma

Fig 2
Early midphase fluorescein angiogram of disc area and part of the ... lower temporal quadrant of left eye (Case No 1) ...

Fig ...
Central part of fundus of the left eye in case No 2 with a ...
White sheathings of some of the vessels

2



3



Case Reports

Case 1 (52 08 14)

A woman aged 19 years was admitted to the Department of Ophthalmology [illegible] under the diagnosis of phacomatosis. Previously in good health and [illegible] eye diseases the patient one month before the admission suddenly [illegible] reading with the left eye. Since then the reduced vision in the [illegible] unchanged.

Objectively normal external eye conditions were found. Left pupil [illegible] the right, both reacted normally to light. Visual acuity was 1.0 in the [illegible] eye. Tension 14/12 mmHg. Normal visual fields. Normal ophthalmoscopy [illegible] right eye. In the left eye ophthalmoscopy showed dilated and tortuous [illegible] fundus and in the lower quadrants just below the disc a tumour [illegible] and highly tortuous vessels forming loops with a 2–3 diopters prominence [illegible] and veins could not be differentiated. No pulsation was seen. In the macula [illegible] DD haemorrhage and in the lower temporal quadrant two [illegible] scattered small haemorrhages. Fluorescein angiography showed rapid [illegible] no dye leakage (Fig. 2).

Fig. 4



Figs. 4, 5 & 16

Early, mid and late phase fluorescein angiogram of the area with part [illegible] Rapid filling of all vessels in the haemangioma and in F[illegible] border of the tumour and in the macula area





Neurological examination revealed hyperactive reflexes. EEG was (irregular 5–7 Hertz activity temporally on both sides). Radiography of ... foramen and left orbit was normal. Left carotid angiography and ... angiography showed no vascular malformations in the brain ... normal. The patient was seen in this department in 1973 and at the ... January 1979 vision and ophthalmoscopy were unchanged. ... angiography had been performed in 1972 and found normal. CT ... performed at the last examination. No treatment was offered.

Case 2 (26 06 20)

A woman aged 48 years previously of good health and suffer... day before admission in November 1974 she experienced a sudden ... eye.

Objective examination showed moderate blepharochalasis and ... Left eye fissure was a little bigger than right. No exophthalmus was present. Visual acuity in left eye 0.33, right eye 1.0. Ophthalmoscopy ... and hypoplastic disc and just above this a tumour consisting ... vessels forming prominant loops (Fig. 3). Arteries and veins could not be ... clearly and no pulsation was seen. From the disc three large dilated ... sheated vessels passed through the vessel conglomerate into the upper ... Scattered in the fundus some small haemorrhages were seen and in the lower ... there was a considerable vitreous haemorrhage. Visual field examination showed ... corresponding to the haemorrhage. Normal ophthalmoscopic find... Fluorescein angiography showed rapid filling of all vessels in the ... some dye leakage in the retina at the temporal border of the tumour and ... (Figs. 4, 5, 6). Radiography of the skull and chest and general examination ... abnormal.

The following years this patient had 7 or 8 periods with slight pain and ... of vision in the left eye. At these periods the patient's ophthalmol... and sometimes slight haemorrhage in the vitreous, and treated the patient ... steroids. According to the ophthalmologist the haemangioma was unchanged ... haemorrhage in the vitreous appeared in September 1978 with severe loss of ...

The patient was again admitted to the department in December ... eye was 1.0 and in the left eye vision was reduced to finger movement ... before, a little larger than the right. No exophthalmus was present. ... both eyes and the pupils were normal. At slit lamp examination ... haemorrhage was seen in the left eye. The fundus could not be seen ... Normal retina in the right eye. CT scanning of the orbits and the brain ... abnormal. No treatment was offered.

Fig. 7

Upper temporal part of fundus of the left eye in case No. 3 ... group 2 racemose haemangioma.

Fig. 8

Mid phase fluorescein angiogram of macula area of the left eye ... dye leakage and abnormal vessels.



Fig 7



Fig 8

Case 3 (36 01 02)

A woman aged 35 years was admitted to the Department of O[illegible] 1971 Previously she had been healthy except for a subconjunctival haemorrhage [illegible] eye at the age of 19 years During the year prior to admission [illegible] decreasing vision in the left eye Three months earlier there was a sudden [illegible] the vision of the left eye

Objectively normal external eye conditions and pupils were found [illegible] the right eye and 0 3 in the left eye Normal ophthalmoscopy in the right eye [illegible] dilated and tortuous vessels were seen in the entire fundus especially [illegible] quadrant where the vessels formed a small conglomerate two DD [illegible] and no pulsation of the vessels was seen no exudates and no haemorrhages [illegible] vessels in the upper temporal quadrant had a whitish sheathing [illegible] examination showed a central scotoma and several scattered, small [illegible] angiography showed rapid filling of the conglomerate of vessels in [illegible] quadrant and dye leakage in the macula area (Fig 8) Neurological [illegible] EEC radiography of the skull was normal Radiography of the left orbita [illegible] thinning in the back wall of orbita Therefore a left sided carotid [illegible] performed but it was normal without any signs of intracranial haemangioma [illegible]

At the follow up examination in January 1979 the patient indicated [illegible] left eye There were no other ophthalmological or neurological [illegible] eye 0 4 in the right eye 1 0 Normal retina in the right eye unchanged [illegible] left eye Carotid angiography was performed in 1971 and found to be normal [illegible] CT scanning of the brain was not performed at this examination [illegible]

Discussion

The ophthalmoscopic findings in the two cases with a localized [illegible] composed of large tortuous vessels is the one most often reported [illegible] deterioration of vision which appeared in two of the patients is a [illegible] manifestation of the racemose haemangioma. On basis of the ophthalmoscopic [illegible] angiographic pictures and of growth pattern different classifications [illegible] suggested (Rundles & Falls 1951 Cameron 1958 Reese 19[illegible] [illegible] al (1973) studying structural abnormalities and alterations in retinal [illegible] dynamics by suction-cup ophthalmodynamometry combined with [illegible] angiography classified three groups of arterio-venous communications [illegible] group is characterized by the interposition of small caliber arteri[illegible] munications between the major vessels. It remains stationary does not [illegible] retinal haemodynamics or the vision

The second group includes arterio-venous anastomosis of larger [illegible] without aneurysmal dilations The venous side of the communication [illegible] terized by hyperdynamic flow and high intraluminar pressure [illegible] exudates oedema venous thrombosis and haemorrhages In the [illegible] arterio-venous anastomoses are of very large caliber and aneurysmal [illegible]

involved vessels which become convoluted and intertwined Some of the is develop fibrous sheatings The venous side of the system suffer from high uminar pressure causing retinal haemorrhages and oedema with severe loss of i. The patients in this group are claimed to have a higher incidense of ranial arterio-venous aneurysms than the normal population Using this fication case No 1 and No 2 should be placed in the third group and case No he second group

sides the complications mentioned above other symptoms have been des-d exophthalmus strabismus anisocoria (as in case No 1) ptosis and gmus (Baurmann et al 1968) The association with intracranial arterio-venous rysm is a well known and controversial subject The co-existence was par irly emphasized by Wyburn Mason (1943) who found a very high incidense) of signs of intracranial aneurysms in the midbrain in patients with retinal nose haemangioma Bech & Jensen (1961) pointed out in a review of the ture that this figure was much too high and considering the relationship een the aneurysms of the two regions from the reverse angle showed that only of the patients with verified cerebral aneurysm had racemose haemangiomas eir retinas This has been confirmed in later studies (Cagianut 1962 Unger & ach 1966)

is now recommended that patients with racemose haemangioma in the retina ld be examined neurologically and if possible a CT-scanning of the brain and s should be done Only if these examinations raise suspicion of intracranial ormations should further neuroradiological examinations be performed None e cases in this paper showed signs of intracranial haemangiomas at cerebral ographs or CT scanning

nong the conditions to be considered in the differential diagnosis most authors tion von Hippel s disease (Angiomatosis retinalis) and von Hippel Lindau s se (Angiomatosis cerebro-retinalis) The major features in these syndromes are ute tumours in the retina with large afferent and efferent vessels and no nunications between the vessels In the Sturge Weber Dimitri disease the al veins may resemble those of the racemose haemangioma but the chorioidal ges the naevus flammeus and the absence of arterio-venous anastomosis may elpful in the differentiation In some diseases vascular communications may r as a result of prolonged vascular stasis e g arterial embolism chronic coma, central venous thrombosis retinal haemorrhage or after chorioretinitis hese conditions are rarely confused with true haemangiomas

he course of this disease varies a great deal from case to case The first ifestations of the racemose haemangioma usually occurs in the second and l decades of life If the haemangiomas are rather small (group 1 or 2 in the ier classification) they probably do not show any or only slight signs of

progression as in case No 1 and No 3 although signs of [illegible] may occur from time to time In a few cases spontaneous [illegible] reported (Gregersen 1966) The prognosis of the large haem[illegible] ally poor as in case No 2

Owing to the good prognosis in most cases treatment is [illegible] selected cases Different surgical methods have been tried [illegible] 1976) In some recent papers photocoagulation has been [illegible] patients with leakage exudates or haemorrhage and conse[illegible] reduced vision where no signs of spontaneous resolution have [illegible] et al 1968 Archer et al 1973) This treatment has however only [illegible] few patients in order to close the abnormal communication or reduce [illegible] afferent artery Generally photocoagulation of such large vessels [illegible] dangerous with risk of severe haemorrhages Considering the [illegible] indications and the hazards of this therapy photocoagulation treatment [illegible] not to be indicated in case No 1 and No 3 In case No 2 photocoag[illegible] a practical proposition at the present time because of the [illegible] blurring the insight to the retina

References

Archer D B Deutman A Ernest J T & Krill A F (1973) Arteriovenous [illegible] the retina *Amer J Ophthal 75* 224–241

Baurmann H Meyer F & Oberhoff E (1968) Komplikationen bei [illegible] Anastomose der Netzhaut *Klin Mbl Augenheilk 151* 5[illegible]

Bech K & Jensen O A (1958) Racemose haemangioma of the retina [illegible] 769–781

Bech K & Jensen O A (1961) On the frequency of coexisting racemose haem[illegible] the retina and brain *Acta psychiat Scand 36* 47–5[illegible]

Cagianut B (196?) Das arterio-venose Aneurysma der Netzhaut [illegible] 180–191

Cameron M E (1958) Congenital arterio-venous aneurysm of the retina [illegible] 655–666

Ehlers H (1924) Aneurysma recemosum arteriovenosum retinae [illegible] 371–387

Frandsen A D (1950) A case of haemangioma retinae *Acta [illegible]*

Gregersen E (1961) Arterio-venous aneurysm of the retina *Acta [illegible]*

Reese A B (1976) *Tumours of the eye* p 27?–?76 3rd ed Harper & Row New [illegible]

Rundles W Z jr & Falls H F (1951) Congenital arterio-venous (racemose) [illegible] retina *Arch Ophthal (Chicago) 46* 408–418

Speiser P (1978) Das arteriovenose Hamangiom der Netzhaut [illegible]

Unger H H & Umbach W (1966) Kongenitales okulo-zerebrales Ra[illegible] *Augenheilk 148* 672–6??

Wyburn Mason R (1943) Arterio-venous aneurysm of midbrain and retina [illegible] mental changes *Brain 66* 163–203

Author's address Peter Bernth Petersen Department of Ophthalmology Århus Kommunehospital DK 8000 Århus C Denmark

Department of Ophthalmology (Head S ·en Erik G \ lson)
Linkoping L nivers ty Sweden

AGE VARIATIONS IN NORMAL HUMAN CONTRAST SENSITIVITY

BY

GUNILLA DEREFELDT GUNNAR LENNERSTRAND and BJÖRN LUNDH

The visual contrast sensitivity (the reciprocal of contrast threshold) was stud ed as a function of age Psychophysical measurements of binocular and mon ocular contrast thresholds were made for 33 normal observers at spatial frequencies within the range 0 5 to 40 cycles degree The observers were divided into three different age groups young middle aged and old subjects with the age ranges 6–10 years 20–40 years and 60–70 years respectively All observers had *healthy eyes normal vision and Snellen visual acuity of 1 0 or* better in both eyes

In all groups contrast sensitivity for binocular and monocular viewing peaked at a spatial frequency around 3–5 cycles degree and showed the typical attenuation at low and high spatial frequencies The binocular contrast sensitivity was higher than the monocular

There was no significant difference between young and middle aged subjects with regard to contrast sensitivity Subjects aged 60 years or more showed significantly lower contrast sensitivity than younger subjects for most spatial frequencies above 4 cycles degree We may thus conclude that both the binocular and monocular contrast sensitiv ty seemed independent of age within the range of 6 to 40 years For h gher ages studied (above 60 years) there was a loss of sensitivity in the middle and high frequency regions.

Key wo ds human contrast sensitivity – sinusoidal gratings – age dependence

ermination of contrast sensitivity to sinusoidally modulated gratings (Campbell reen 1965 Campbell 1973) has become clinically important as a method of luating visual dysfunction (Arden 1978) This psychophysical method has been

e ed February 1 1979

employed in studies on the visual effects of optical derangement [illegible] edema (Hess & Garner 1977) and cataract (Hess & Woo 197[illegible] [illegible] Jacobson 1978) visual dysfunction in macular disease (Sjostrand & [illegible] optic neuritis (Regan et al 1977 Frisen & Sjostrand 19[illegible] [illegible] 1978) in meridional amblyopia (Mitchell et al 1973 Freeman & [illegible] anisometropic or strabismic amblyopia (Spekreijse et al 19[illegible] Hess & [illegible] Hess & Smith 1977 Levi & Harwerth 1977 Sjostrand 19[illegible] disturbances due to cortical lesions (Bodis Wollner 19[illegible] 1976 [illegible] Diamond 1976)

Thus many pathological states have been examined but the results have [illegible] been related to only a few normal subjects or subjects not differentiated [illegible] to age in each investigation In a study by Arundale (1978) on the [illegible] of contrast sensitivity only a few young and old subjects were examined [illegible] Jacobson (1978) found a lower sensitivity in subjects over 40 years [illegible] younger subjects but the range of spatial frequencies tested was rather [illegible]

As a basis for clinical studies monocular and binocular contrast [illegible] measured in young middle aged and old subjects with normal vision The [illegible] thresholds were determined for a wide range of spatial frequencies [illegible] tional techniques but employing a higher luminance level than [illegible] previous studies

Materials and Methods

Methods

The visual stimuli presented were vertical gratings displayed on a [illegible] oscilloscope (Tektronix 5440 with P31 phosphor) By means of a [illegible] signal from a function generator (Tektronix FG 501) an even [illegible] was produced The beam was intensity modulated with another [illegible] (Exact 129) producing a sinusoidal luminance profile on the screen The [illegible] contrast could be varied continuously by means of an attenuator The [illegible] oscilloscope was sensed by a peak detector and the contrast value was [illegible] displayed Contrast was defined as $L_{max} - L_{min}/L_{max} + L_{min}$ The maximum [illegible] used was 0 13 The display subtended 4 5 × 5 0 degrees of visual [illegible] viewing distance of 143 cm which was kept constant by means of a [illegible] chinrest The mean luminance of the screen was 1 0 cd/m² The [illegible] illuminated by an ordinary desk lamp containing a 60 W incandescent [illegible] surround luminance of about 20 cd/m² The subject was adapted to [illegible] for at least 10 min before the experiment

ects

…n three normal observers with ages in the range 6 to 70 years participated in …investigation They were divided into three age groups one of 10 subjects …een 6 and 10-years-old (8 girls and 2 boys) one of 12 subjects between 20 and …ears-old (10 women and 2 men) and one of 11 subjects of 60 years or older (8 …en and 3 men) A smaller group of 5 subjects (3 men and 2 women) between 40 …60-years-old was also examined No one had any previous experience in …shold experiments Eyes and vision were normal in all subjects as revealed by …thalmological examination including careful refraction and binocular assess…nt. All subjects had Snellen visual acuity of 1 0 or better in both eyes and could …ect stereoscopic disparities down to 40 sec of arc Subjects above 60 years-of age …e given + 0 5 sph in addition to their static refraction in order to compensate … the viewing distance Normal pupils were used

cedure

…e contrast thresholds were determined binocularly and for each eye alone at 19 …ial frequencies ranging between 0 5 and 40 cycles degree The threshold was …ermined by raising the contrast from a subthreshold value at a selected spatial …quency until a grating pattern was just detected on the screen The contrast was …ied continuously by the experimenter by means of a potentiometer We have …d this most commonly employed method and not the reverse procedure i e …ering the contrast from a supraliminal level which might induce disturbing …ating adaptation phenomena (Blakemore & Campbell 1969) The training of the …bjects before the experiment included presentations of gratings well above …reshold contrast in the low middle and high frequency regions and a few …reshold determinations Intermittently during the testing the subjects were asked … give an estimate on the grating density of the pattern which could just be …tected This was used as a control of their reports on pattern recognition The …atial frequencies were presented in a random order The initial sessions were …rformed in the middle aged subjects who in binocular viewing made five …dgements per frequency The values were very consistent for each subject and …r the rest of the binocular testings three judgments per frequency were con…dered sufficient. During the monocular viewing all subjects made two judgements …r each frequency For the binocular viewing the session lasted about one hour for …ach subject in the middle aged group and about half an hour in the two other …roups For the monocular viewing condition the session lasted approximately 20 …in for each eye During the monocular testing the other eye was covered with a …lack occluder The binocular and monocular testing was mostly carried out on …eparate occasions Four different experimenters have been involved in the testings …ach examining subjects of at last two age groups

Results

For each subject the contrast sensitivity (the reciprocal of the mean [illegible] contrast values) was plotted against spatial frequency for [illegible] viewing Examples of individual curves typical of each age group [illegible] The curves characterising the contrast sensitivities were quite [illegible]

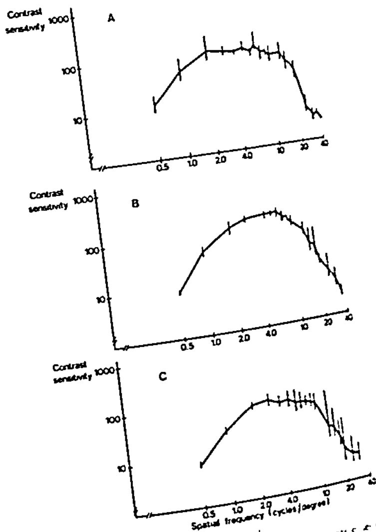


Fig 1

Monocular contrast sensitivity of an eight year-old boy (A) a [illegible] and a sixty-one year-old woman (C) plotted against spatial frequency [illegible] Data points are the reciprocal of the arithmetical mean of four threshold [illegible] (two for the left and two for the right eye) Bars represent ± 1 [illegible]

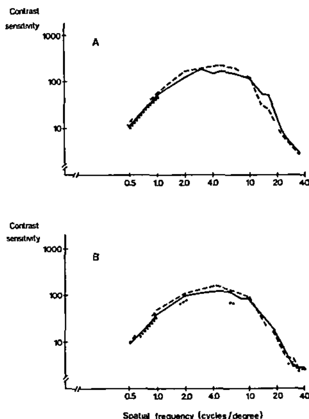


Fig 2

Binocular contrast sensitivity for three age groups (———— 6–10 years 20–40 ars 60–70 years) plotted against spatial frequency in log log coordinates Data points are the reciprocal of the average binocular contrast thresholds for all individuals in each group (see Table I)
The same as in A but for monocular contrast sensitivity

the children The variations between measurements at each spatial frequency was about the same at all ages as seen in Fig 1 The curves most often peaked at spatial frequencies of 3–6 cycles/degree They were typically attenuated at low and high frequencies They were very similar for the right and the left eye The monocular contrast sensitivity given in Fig 1 is therefore an average value of the results from both eyes The binocular contrast sensitivity was generally higher than the monocular one

The contrast sensitivity (binocular and monocular) for each age group is plotted against spatial frequency in log log coordinates in Fig 2 For all groups the curves had a peak sensitivity at a spatial frequency around 3–5 cycles/degree Contrast

Table I

Binocular threshold contrast (means and SD s determined for [illegible] from 0.5 to 40 cycles/degree The contrast sensitivity (reciprocal [illegible] contrast) is also given The values are given for each age group No 1 [illegible] 2 = 20–40 years No 3 = 60–70 years) The F ratios from the analysis [illegible] for each spatial frequency

Spatial frequency cycles/degree	Age group	Threshold contrast		Contrast sensitivity	Number of subjects	F
		M	SD			
0.5	1	0.1003	0.0221	9.97	10	[illegible]
	2	0.09[illegible]0	0.0727	10.53	1[illegible]	P > [illegible]
	2	0.1075	0.0318	9.30	11	
1.0	1	0.0194	0.0075	51.4[illegible]	13	[illegible]
	2	0.0188	0.0095	53.31	1[illegible]	P [illegible]
	3	0.0227	0.0087	44.15	11	
2.0	1	0.0081	0.0039	1[illegible]3.61	13	[illegible]
	2	0.0065	0.0018	154.84	1[illegible]	P [illegible]
	3	0.0100	0.0033	100.03	11	
3.0	1	0.0056	0.0017	1[illegible]7.31	13	[illegible]
	2	0.0052	0.0011	191.3[illegible]	1[illegible]	P >
	3	0.0067	0.001[illegible]	149.46	11	
4.0	1	0.0067	0.0027	150.15	10	[illegible]
	2	0.0047	0.0013	[illegible]1.77	1[illegible]	P <
	3	0.0076	0.0024	131.58	11	
5.0	1	0.0058	0.0014	1[illegible]41	13	[illegible]
	2	0.0045	0.0010	[illegible]1.00	1[illegible]	P < [illegible]
	3	0.0080	0.0020	1[illegible]5.00	11	
6.0	1	0.0069	0.0020	16[illegible]	13	[illegible]
	2	0.0043	0.001[illegible]	[illegible]03.[illegible]4	1	P <
	3	0.0091	0.0028	109.45	11	
7.0	1	0.0064	0.0016	155.[illegible]8	13	[illegible]
	2	0.005[illegible]	0.0011	133.55	1	P <
	3	0.0102	0.0031	[illegible]7.[illegible]	11	
8.0	1	0.0072	0.0022	133.08	13	[illegible]
	2	0.0058	0.0014	1[illegible]5.16	1[illegible]	P <
	3	0.0117	0.0029	85.[illegible]1	11	
9.0	1	0.0075	0.0024	13[illegible].63	13	
	2	0.0067	0.0018	14[illegible].[illegible]0	1	P [illegible]
	3	0.0145	0.0043	[illegible]9.84	11	

Table I (cont.)

atial uency /degree	Age group	Threshold contrast		Contrast sensitivity	Number of subjects	F
		M	SD			
0 0	1	0 0082	0 0027	122 10	10	11 32
	2	0 0085	0 0026	117 07	12	$P < 0\ 01$
	3	0 0165	0 0071	60 71	11	
14 0	1	0 0179	0 0083	55 93	10	3 38
	2	0 0291	0 0277	34 33	12	$P > 0\ 01$
	3	0 0478	0 0352	20 91	11	
16 0	1	0 0190	0 0068	52 77	10	12 41
	2	0 0373	0 0262	26 82	12	$P < 0\ 01$
	3	0 0883	0 0505	11 32	11	
18 0	1	0 0506	0 0548	19 77	10	3 63
	2	0 0592	0 0420	16 90	12	$P > 0\ 01$
	3	0 1248	0 1007	8 02	11	
20 0	1	0 0749	0 0571	13 34	10	7 46
	2	0 0867	0 0531	11 54	12	$P < 0\ 01$
	3	0 1599	0 0567	6 26	11	
25	1	0 1633	0 0541	6 12	10	2 77
	2	0 1706	0 1070	5 86	11	$P > 0\ 01$
	3	0 2474	0 0856	4 04	9	
30 0	1	0 2498	0 1089	4 00	10	1 21
	2	0 2360	0 0843	4 24	8	$P > 0\ 01$
	3	0 3363	0 0836	2 97	3	
35 0	1	0 3146	0 0693	3 18	7	1 31
	2	0 3043	0 0899	3 29	4	$P > 0\ 01$
	3	0 4300		2 33	1	
40 0	1	0 2117			1	

sitivity curves of men and women showed no differences within each age group contrast sensitivity and the group means of the monocular and binocular trast thresholds are given in Tables I and II for each age group Both the ocular and the monocular data show that the old age group has a lower contrast sitivity i e higher contrast thresholds for all spatial frequencies compared to two younger age groups As shown by an analysis of variance and t tests of the

Table II

Monocular threshold contrast (means and SDs determined for 19 [illegible] from 0.5 to 40 cycles degree The contrast sensitivity (recip[illegible] contrast) is also given The values are given for each age group No 1 [illegible] 2 = 20–40 years No 3 = 60–70 years) The F ratios from the anal[illegible] for each spatial frequency

Spatial frequency cycles/degree	Age group	Threshold contrast		Contrast sensitivity	[illegible]	[illegible]
		M	SD			
0.5	1	0.1051	0.0971	9.5[illegible]	13	[illegible]
	2	0.0997	0.0273	10.03	13	P<
	3	0.1418	0.0391	[illegible]	11	
1.0	1	0.0213	0.0101	41.53	13	[illegible]
	2	0.0205	0.0101	48.56	10	P<
	3	0.0?72	0.0077	[illegible]	11	
2.0	1	0.0096	0.0017	104.55	10	[illegible]
	2	0.0085	0.0027	[illegible]	13	P<
	3	0.0123	0.0027	81.5	11	
3.0	1	0.0085	[illegible]	117.10	10	[illegible]
	2	0.0072	0.0015	[illegible]	10	P<
	3	0.0109	0.0026	91.[illegible]	11	
4.0	1	0.0083	0.0034	[illegible]	13	[illegible]
	2	0.0063	0.0013	[illegible]	13	P
	3	0.0102	0.0021	[illegible]	11	
5.0	1	0.0050	0.0023	[illegible]	13	[illegible]
	2	0.0062	0.0017	161.5[illegible]	10	P<
	3	0.0120	0.0024	85.05	11	
6.0	1	0.0083	0.0027	[illegible]	13	[illegible]
	2	0.0071	0.0015	141.84	10	P<
	3	0.01[illegible]6	0.0033	[illegible]	11	
7.0	1	0.0090	0.0021	111.56	13	
	2	0.0074	0.0015	135.[illegible]9	13	P<
	3	0.0141	0.0045	69.55	11	
8.0	1	0.0106	0.00[illegible]3	[illegible]	10	[illegible]
	2	0.0084	0.0017	118.0[illegible]	13	P<
	3	0.0167	0.0065	60.00	11	
[illegible]	1	0.0111	0.003	[illegible]	13	[illegible]
	2	0.0099	0.00[illegible]5	[illegible]	13	P<
	3	0.0191	0.0064	[illegible]	11	

Table II (cont)

Spatial frequency c/s/degree	Age group	Threshold contrast		Contrast sensitivity	Number of subjects	F
		M	SD			
10 0	1	0 0125	0 0038	79 75	10	13 16
	2	0 0115	0 0096	86 73	10	$P < 0\ 01$
	3	0 0240	0 0095	41 69	11	
14 0	1	0 0281	0 0167	35 61	10	5 85
	2	0 0373	0 0399	96 80	10	$P < 0\ 01$
	3	0 0833	0 0568	12 00	11	
16 0	1	0 0385	0 0281	25 98	10	8 5
	2	0 0468	0 0340	21 37	10	$P < 0\ 01$
	3	0 1385	0 0951	7 29	11	
18 0	1	0 0531	0 0228	18 84	10	11 57
	2	0 0615	0 0466	16 26	10	$P < 0\ 01$
	3	0 1728	0 0961	5 79	11	
20 0	1	0 0925	0 0322	10 81	10	7 12
	2	0 1231	0 0695	8 13	10	$P < 0\ 01$
	3	0 1942	0 0749	5 15	10	
25 0	1	0 1948	0 0542	5 13	10	4 10
	2	0 1962	0 1443	5 10	9	$P > 0\ 01$
	3	0 3103	0 1108	3 22	8	
30 0	1	0 3198	0 0906	3 13	10	4 08
	2	0 2145	0 0409	4 66	6	$P > 0\ 01$
	3	0 2518	0 0408	3 97	3	
35 0	1	0 3083	0 0689	3 24	6	1 08
	2	0 3425	0 0538	2 92	5	$P > 0\ 01$
	3	0 3998		2 50	1	
40 0	1	0 3284	0 0716	3 05	5	0 52
	2	0 3670	0 0064	2 73	2	$P > 0\ 01$

reshold values this difference was significant at the 0 01 level (see Table I and II ratios) for most frequencies between 5 0 and 20 0 cycles/degree In the low spatial equency range *(below 4 cycles/degree) this difference was significant (t tests)* tween the old and middled aged subjects for monocular viewing The binocular alues showed no significant differences between age groups in this frequency inge

In the low and the middle frequency region (0 5–10 cycles degree ... sensitivities of the children tended to be lower than those f ... group (Fig 2) At higher spatial frequencies the trend was reversed ... shown by t tests there was no significant difference between the two ... groups at any spatial frequency In the highest spatial frequency range ... above) no significant differences were seen between any of the ... few old subjects were able to detect the gratings at the highest f... Tables I and II)

As a control of these results we have tested five subjects of age ... 60 years They showed contrast sensitivity curves with slightly lower ... 20–40 year old subjects in the higher spatial frequency range ...

In order to compare monocular and binocular contrast sensitivity ... average monocular to binocular threshold was determined at all ... for each individual The variations of monocular to binocular ratio ... frequency were larger for our subjects than for those tested by Campbell ... (1965b) and Blake & Levinson (1977) However average values for each ... showed much smaller fluctuations (Fig 3) The arithmetic mean of the ... for all spatial frequencies between 0 5 and 30 cycles degree was ... the youngest 1 43 ± 0 16 SD for the middle and 1 45 ± 0 18 SD for the ... group These values are almost the same as those obtained by Campbell ... (1965b) and Blake & Levinson (1977) for similarly aged subjects ... individual and the group data we found a tendency for the ratio to ... spatial frequency (Fig 3)

At subthreshold contrast some subjects reported seeing a grating ... spatial frequency than that displayed Most often they described a ... pattern while the tested frequency could be of any value Sometimes ... frequency were reported during the testing at a high frequency ... explanation for this phenomenon

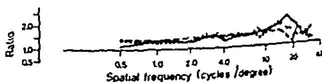


Fig 3

The ratio of average monocular to binocular threshold for three age groups ... year ... 0–40 year ... 60–70 year ... and 30 cycles degree Data points are the arithmetic mean of the ... frequency for all individuals in the age group

Discussion

findings are in close agreement with existing data with respect to the general e of the contrast sensitivity curve In all age groups the curve peaked in the uency range of 3–5 cycles/degree and showed the typical attenuation in the low high spatial frequency regions Contrast threshold was quite easily defined even ar youngest subjects

is study has shown that the contrast sensitivity is dependent on age For cts in our oldest age group (60–70 years) we found a decline in sensitivity to ings in the middle and high spatial frequency regions that was not seen in ger persons All subjects below 40 years of age had very similar contrast itivity curves At ages between 40 and 60 years there was a gradual shift in rast sensitivity towards the values of the old subjects

he loss of sensisivity for high spatial frequencies found in the oldest persons is in eement with the data of Arundale (1978) It might at least partially be ained as an effect of senile miosis (Woodhouse 1975) Our young subjects did show the reduction in sensitivity to low spatial frequencies reported by ndale (1978) It should be noted however that only three eyes of subjects in the group 8–15 years were tested by Arundale and that the mean Snellen acuity of e eyes was 0 8 while all our subjects had a visual acuity of 1 0 or more

he difference between our young and old subjects might be considered quite ll, when compared to those reported in the literature between normal subjects patients suffering from various visual disorders (see Introduction for ref ces) However we conclude that the age of the patient has to be taken into ideration when using the contrast sensitivity determination as a method of uating visual dysfunction especially in borderline cases

Acknowledgments

study was supported by grants from Carmen and Bertil Regner s Research Foundation Research Committee of Östergotlands Lans Landsting Konsul Thure Carlsson s Found and the Swedish Medical Research Council (No 12X 734)

References

en G B (1978) The importance of measuring contrast sensitivity in cases of visual turbance *Brit J Ophthal 62* 198–209

en G B & Gucukoglu A C (19 8) Grating test of contrast sensitivity in patients with r bulbar neuritis *A ch Ophthal (Chicago) 96* 16 6–1629

n G B & Jacobson J J (19 8) A s mple grating test for contrast sensitivity Preliminary sults indicate value in screening for glaucoma *Inv t Ophthal V al Sci 1* 23–32

Arundle K (1978) An investigation into the variation of human ... and ocular pathology *Brit J Ophthal* 62 213–215
Blake R & Levinson E (1977) Spatial properties of binocular neurones ... system *Exp Brain Res* 27 221–239
Blakemore C & Campbell F W (1969) On the existence of neurones in the ... system selectively sensitive to the orientation and size of retinal images *J Physiol* 203 237–260
Bodis Wollner I (1972) Visual acuity and contrast sensitivity in patients ... *Science* 178 769–771
Bodis Wollner I (1976) Vulnerability of spatial frequency channels in ... *Nature* 261 309–311
Bodis Wollner I & Diamond S P (1976) The measurement of ... cases of blurred vision associated with cerebral lesions *Brain* 99 695–)
Campbell F W (1973) The transmission of spatial information through ... In Worden F C & Schmidt F O eds *The Neurosciences* ... MIT Press Cambridge Mass
Campbell F W & Green D G (1965a) Optical and retinal factors affecting ... *J Physiol (Lond)* 181 576–593
Campbell F W & Green D G (1965b) Monocular versus binocular visual ... 191–192
Freeman R D & Thibos L N (1975) Contrast sensitivity in humans ... experience *J Physiol (Lond)* 247 687–710
Frisen L & Sjostrand J (1978) Contrast sensitivity in optic neuritis ... 17 165–174
Hess R F & Carney L F (1977) The effect of corneal edema on visual ... *Ophthal Visual Sci* 16 5–13
Hess R F & Howell E R (1977) The threshold contrast sensitivity function in amblyopia Evidence for a two type classification *Vision Res* 17 1049–1055
Hess R F & Smith C (1977) Do optical aberrations contribute to visual loss in amblyopia *Amer J Optom* 54 627–633
Hess R F & Woo C (1978) Vision through cataracts *Invest Ophthal Visual Sci* ...
Levi M & Harwerth R S (1977) Spatio-temporal interactions in ... strabismic amblyopia *Invest Ophthal Visual Sci* 16 90–95
Mitchell D E Freeman R D Millodot M & Haegerstrom G (1973) Meridional ... Evidence for modification of the human visual system by early visual experience ... 13 535–558
Regan D Silver R & Murray J T (1977) Visual acuity and contrast sensitivity in multiple sclerosis hidden visual loss An auxiliary diagnostic test *Brain* 100 563–579
Sjostrand J (1978) Contrast sensitivity in amblyopia A preliminary report *J* ... 13 135–137
Sjostrand J & Frisen L (1977) Contrast sensitivity in macular disease *Acta Ophthal (Kbh)* 55 507–514
Spekreijse H Khoe L H & van der Tweel L H (1972) A case of amblyopia ... physiology and psychophysics of luminance and contrast In Arden G B ed *The Visual System Neurophysiology Biophysics and their Clinical Applications* ... Plenum New York
Woodhouse J M (1975) The effect of pupil size on grating detection at various contrast levels *Vision Res* 15 645–648

Authors' address ... C Derefeldt Th D C Lennerstrand M D and B L... M ...
Department of Ophthalmology University Hospital S-... Linköping Sweden

ACTA OPHTHALMOLOGICA VOL 57 1979

Department of Ophthalmology (Head \ Ehlers)
and Department of \uclear Med cine I otope L nit Radiumcentre
(Head H Hud Hansen)
Irhus Kommunehospital L nr er its of Aarhus Denmark

CONJUNCTIVAL TRANSPORT OF TECHNETIUM 99m PERTECHNETATE

BY

T SØRENSEN and F TAAGEHØJ JENSEN

Lsin, a gamma camera coupled to a computer and a tape unit the transport of pertechnetate (Tc^{99m}) across the conjunctiva was determined by the region of interest *technique in human subjects*

In 11 patients with their lacrimal sacs removed a fractional turnover rate of 0 0?1 min $^{-1}$ was found In 21 patients with inflammed conjunctiva due to chronic dacryocystitis a fractional turnover rate of 0 0?/ min $^{-1}$ was found whereas the alue in 11 normal individuals was 0 01? min $^{-1}$

\e lecting the relatively small transcorneal route of disappearance the alues found for technetium disappearance could be regarded as representati e for the transconjunctival transport of the radioisotope Thi a sumption was confirmed by correlating the radioactivity in the blood with the fractional turnover rate

Key words conjunctival transport – radioactive tracer gamma camera – human individuals – dacryocystitis

? little attention has been paid to the transport of solutes bet een the lacrimal d and the blood across the conjunctiva Though it is well known that atropine l other substances can influence other structures than the eye after instillation in conjunctival sac due to absorption to the general circulation quantitative data this subject are few

t seems that Schirmer (1903) was the first to measure the disappearance of drugs m the conjunctival sac He introduced salicylic acid into the eye of a patient with / exstirpated lacrimal sac and found a five hundred fold decrease in concen

eived June 3 19/8

trition in 1½ h which is equivalent to an 8.3% loss in one min if ... elimination is assumed

In a study on permeability of excised bovine corneas ... that the conjunctiva sclera combination was a selective barrier ... substances as was the corneal epithelium stroma combination

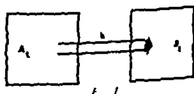
The first report on conjunctival transport using radioactive ... Ussing (1967) who found a disappearance rate (= fractional ... 0.005 min⁻¹ (0.0001 min⁻¹ cm⁻²) in rabbits with closed canaliculi ... the fractional turnover rate of Na^{22} in anesthetized animals. The same ... found when the cornea was protected by a suction cup

Similar experiments have been carried out by Maurice ... of permeability to sodium and chloride about ten times higher than ... The methods were not strictly comparable because Maurice formed a basin by pulling up the lids with clips – a procedure not used by Ussing ... the canaliculi surgically and left the eyelids untouched during ...

In human beings the first results were presented by Meyer & ... They stated in a preliminary report that 30% of the technetium had ... from the conjunctival sac after 15 min corresponding to a fractional ... of 0.024 min⁻¹. Similar measurements were presented at a meeting ... by Denffer & Dressler (1975). Their material comprised ... block of the tear pathways. In these patients 23.2% of the ... pertechnetate in saline solution) had disappeared after 15 min ... fractional turnover rate of 0.0176 min⁻¹.

The present investigation was originally started in an attempt to ... background radiation in tear flow determinations (Sørensen & ... 1975, 1977). However it soon became evident that the transport ... could be surprisingly high

In this paper the transport of technetium – as pertechnetate ... across the conjunctiva has been measured in patients suffering ... dacryocystitis (group I), in patients with extirpated lacrimal sac ... normal individuals (group III).



Model for calculation of fractional turnover rate. A_t is the activity at ... time t, B_t ... the total activity in the body at time t and k is the fractional ...

al

. radioactive tracer is introduced in the conjunctival sac in patients without the normal ...es for tear outflow the biological system can be simplified as shown in Fig 1 Assuming exponential elimination from the conjunctival sac the decrease of activity in the ...nctival sac follows equation (1)

$$A_t \quad A_o e^{\ k} \qquad (1)$$

...he increase of activity in the body equation (2)

$$B_t = A_o (1 - e^{\ k}) \qquad (2)$$

...e A_o and A_t are the radioactivity at time o and t respectively

B_t the radioactivity at time t in the body

e the base of the natural logarithm and

k the fractional turnover rate

...retransport of technetium from the general circulation to the tears has been considered ...all that it has been ignored in the calculation

Material

...study comprised 43 patients admitted to the hospital for dacryocystitis or for ...ocupational purposes The dacryocyst-exstirpated patients (group II) had had ...lacrimal sacs removed at least several months before the present investigation ...eyes of these patients were without inflammation The normal individuals ...went a tearflow determination as described in another paper (Sørensen & ...høj Jensen 1977) At the end of this determination no radioactivity had ...ed the nose This is to be expected in some normal persons since a primary ...s test will only be positive in approximately 80% of normal eyes (Zappia & ...er 1972) None of the normal volunteers suffered from watery eyes. With a ...on of interest including the conjunctival sac and the tear pathways the ...ppearance of radioactivity represented the transport to the blood The normal ...ons were younger (20–30 years of age) than the dacryocyst-exsturpated patients ...60 years of age) The patients with dacryocystitis were 18–70 years old The ...nal individuals were included in the normal material for tear flow studies (to be ...ished)

Method

...tailed descriptions dosimetric calculations detection system and background radiation ...ørensen & Taagehøj Jensen (1977)

...e radioactive tracer used was technetium Tc^{99m} as pertechnetate in a normal saline ...ion. The patient was placed in a supine position with the eye approximately 3 cm under

the pinhole collimator in the gamma camera A volume of 10 µl (900 µCi) of the technetium solution was placed on the cornea while the lids were firmly held open No anaesthesia was used

Using the region of interest technique quantitative data of the elimination of activity from the designated area i e the conjunctival sac in dacryocyst-exstirpated patients and the conjunctival sac and tear pathways in normals and patients with dacryocystitis

The measuring time was 15 min Radioactivity was accumulated at 10 sec intervals and the resulting 90 numbers were plotted as an activity time function curve in a semilogarithmic system The fractional turnover rate was calculated for the approximated curve (l in equation (1))

In these experiments the fractional turnover rate represented the amount of activity entering the general circulation per minute Since the counting rates were very high a correction for background components was not necessary

In 18 patients blood samples were taken 18 min after instillation in the conjunctiva the uptake of technetium in the thyroid gland and gastric mucosa was blocked by perchlorate one hour prior to the determination in some of the patients In one patient the radioactivity in blood samples was measured at three min intervals after instillation

Results

In Fig 2 a scintigram from a patient where the lacrimal sac has been removed Fig 3 is from a patient with dacryocystitis To fill the lacrimal sac properly with technetium it was necessary to express the fluid retained in the sac prior to



Fig 2 Fig 3

Fig 2
Scintigrams of a patient with removed lacrimal sac Most of the radioactivity is located in the inner and outer canthal area

Fig 3
Scintigram of a patient with chronic dacryocystitis and retention in the lacrimal sac with most of the radioactivity located in this area A similar picture was seen in the 11 normal individuals (see text)

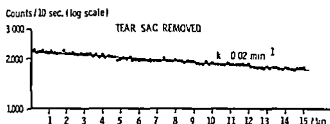


Fig 4

Elimination curve from the conjunctival sac in a semilogarithmic plot
The fractional turnover rate is noted by k

lation In the 11 normal volunteers the scintigrams looked like Fig 3 with no activity in the lacrimal duct.

The elimination curve turned out to be a single straight line in the semilogarithmic plot in all the 43 patients (Fig 4) Thus the assumption of an exponential of elimination was acceptable

The fractional turnover rates in the three groups can be seen in Table I In groups I and III the absorptive area was the conjunctival sac the cornea the canaliculi and the lacrimal sac whereas the absorptive area in group II was the conjunctival sac the cornea and the canaliculi Though the absorptive area was the in groups I and III the transport was significantly higher in group I that included patients with intermittent red eyes (Mann Whitney test 2p = 0 0026) patients with a removed lacrimal sac also had a tendency to a higher transport to the blood compared with the normal individuals though their absorptive area a lacrimal sac less than that of the normal individuals (Mann Whitney test

Table I

Fractional turnover rate and the absorption of technetium after 15 min
(calculated from the fractional turnover rate)
in patients with a stop in the lacrimal passages at different levels
and in normal persons

	Mean fractional turnover rate ± SEM	Fractional turnover rate converted to percentage absorption to the blood
Dacryocystitis n = 21	0 0271 ± 0 0036 min⁻¹	33%
Tear sac removed n = 11	0 0214 ± 0 0040 min⁻¹	28%
Normal individuals n = 11	0 0145 ± 0 0012 min⁻¹	20%

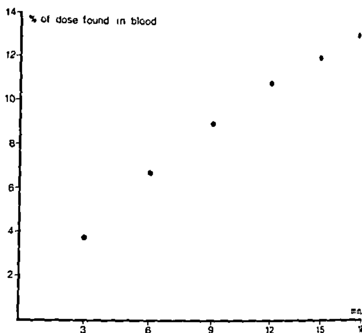


Fig 5
The increase of radioactivity in blood plotted versus time after instillation in the conjunctival sac in one person

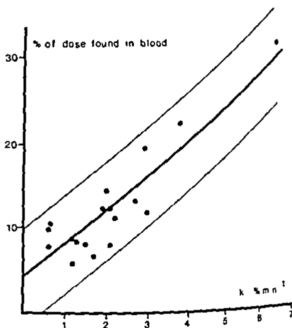


Fig 6
Correlation between the amount of technetium found in the blood 18 min after instillation and the fractional turnover rate in 18 persons (k in the figure)

> 0 10) These normal volunteers did not have a history of watery eyes and they had normal diphasic curves without any sign of obstruction of the tear pathways The fractional turnover rate in groups I and II were not significantly different (= 0 2–0 3)

A confirmation of the transport to the blood is given in Fig 5 The values in the od were not corrected for the leaking of technetium out of the general culation since the magnitude of this transport is relatively unknown in a short m situation like this With this inaccuracy in mind a rough calculation of the rate increase in the blood resulted in a value of the same size as the fractional nover rate in this patient

n Fig 6 the correlation between the fractional ruenover rate and the amount of hnetium found in the blood 18 min after instillation is shown One of these terminations was discarded because for some unknown reason it was more than ee standard deviations from the regression line There was no difference tween the patients premedicated with perchlorate and those with no preme cation In this correlation study patients from all the three groups (Table I) were r

Discussion

lough it has been known for many years that drugs introduced in the conjunc al sac can reach the general circulation most studies on the transport rates in the iter eye have been concerned on the permeability of the cornea Until the works Ursing (1967) and Maurice (1973) only semiquantitative data on conjunctival rmeability were available Unfortunately Maurice found values ten times higher an Ursing probably owing to the greater absorptive area in Maurice s rabbits with conjunctival basin formed by pulling the lids Thus Ursing s method seems most mparable to the *in vivo* conditions found in man When the fractional turnover te in Ursing s paper is corrected for the bigger surface area in man a value of 006 min $^{-1}$ for the positively loaded ion Na^{22} compared to 0 021 min $^{-1}$ found in is work for the negatively loaded pertechnetate Denffer & Dressler (1976) found value of 0 018 min $^{-1}$ in 68 patients with stop in their tear passages Our result in a omparable group of patients was 0 025 min $^{-1}$ (s = 0 015 Mean of group I and II Table I) corresponding to an absorption of technetium to the circulation of 31% 15 min (Denffer & Dressler 23%) This difference can probably be explained by issimilarities in methods In a meeting programme Denffer & Dressler (1975) emonstrated a tendency to a greater absorption the higher the stenosis in the tear athways We found the same tendency with higher fractional turnover rates in atients with removed lacrimal sac and dacryocystitis patients than in normal

Department of Ophthalm logy (Hea I Torsten I Brielen) University of Bergen Bergen Norway

TIMOLOL MALEATE IN TREATMENT OF OPEN ANGLE GLAUCOMA

BY

HENRY AASVED JOHAN H SELAND and JON E SLAGSVOLD

The effect of Timolol maleate on the intraocular pressure in open angle glaucoma has been examined in 37 patients of these simple glaucoma in 26 (48 eyes) capsular glaucoma in 9 (14 eyes) and chronic secondary glaucoma in 2 patients (2 eyes) These cases constituded a group which was relatively difficult to manage

The average pressure reduction caused by Timolol maleate alone was about 23%. In 18 patients the intraocular pressure was adequately controlled on Timolol as the only drug and in 10 on additional drug therapy Five patients failed on drug treatment and the remaining four failed on one eye while the fellow eye was well regulated

Tonography indicates that the effect is caused by a reduction of the aqueous humour production

Side effects of locally applied Timolol maleate have not been observed. This drug may be the drug of choice in many instances

Key words Timolol maleate – beta adrenergic blocking agents – open angle glaucoma – simple glaucoma – capsular glaucoma

Over the course of the last few years several beta adrenergic blocking agents have been introduced in glaucoma treatment Both oral and local application have been proved to reduce the intraocular pressure Such drugs have included atenolol (Brenkman 1976 Wettrell & Pandolfi 1977 Philips et al 1977) propranolol (Phillips et al 1967 Cote & Drance 1968 Musini et al 1971 Vale et al 1972 Wettrell & Pandolfi 1975 Borthne 1976) bupranolol (Krieglstein et al 1976) pindolol

Received January 18 1979

ɔmi & Steindler 197ɔ) oxprenolol (Stilma 1976) and Timolol (Katz et al 1976 alles et al 1977 Zimmerman & Kaufman 1977 Zimmerman et al 1977 ven 19/8 Kerty & Hørven 1978) Among these Timolol maleate seem to have advantages over the others Applied locally the cornea sensibility is unaltered ontrast to propranolol and bupranolol and the effect on the intraocular *sure lasts considerable longer than after application of atenolol pindolol or* enolol In addition Timolol maleate has been used systemically for several s in the treatment of arterial hypertension and angina pectoris without reports rious side effects

or the last two years clinical trials using Timolol maleate have been initiated over areas of the world The intention with this paper is to report our experience locally applied Timolol in the treatment of various types of open angle coma Of special interest is the effect on capsular glaucoma

Material and Methods

s clinical trial comprised 37 patients with open angle glaucoma where simple icoma was found in 26 (48 eyes) capsular glaucoma in 9 (14 eyes) and 2 patients ves) had chronic secondary glaucoma caused by chronic uveitis in one and enital cataract treated surgically in the other Fourteen were inpatients and 23 patients

he one *patient with operated congenital cataract and secondary glaucoma* was ears-old while the age distribution of the remainder of the patients ranged n 46 to 82 years Two patients were new cases (1 simple and 1 capsular icoma) while the others had previously received conventional antiglaucomatous g therapy

rapeutic procedure Prior to the trial all glaucoma medication was stopped After a sh-out period" varying from one to seven days the following examinations were formed Ordinary ophthalmological status including perimetry pupillometry planation *tonometry (Goldmann tonometer) evaluation of tear production* by irmers test and pulse and blood pressure measurements These examinations re repeated during the control period

The aim was to establish an intraocular presure (iop) below 22 mm Hg Initially nolol 0 2ɔ% once a day (at night) was given Depending on the iop level seen at end of the wash out period the pressure was controlled one to seven days later the iop still exceded 22 mm Hg the Timolol medication was increased to 0 ɔ% ce a day and later to 0 ɔ% twice daily if necessary If the iop still exceeded 22 mm

satisfactory pressure regulation Another patient (No 9) had good regulation of simple glaucoma in his left eye on Timolol medication for 5 months, followed by a dramatic rise in pressure refractile to any medication and trabeculectomy was carried out His right eye with secondary glaucoma caused by uveitis was, however, well maintained on Timolol alone as illustrated in Fig 1 These patients (Nos [illegible]) are registered in the column for iop above 22 mm Hg in Table I

The effect of Timolol and additional therapy in 17 patients is seen in Fig [illegible] In these patients Timolol alone was not able to maintain the iop below 22 mm Hg In two patients (Nos 7 & 23) the iop was even higher on Timolol alone than during the wash out period

In 10 of the patients the iop was well regulated on Timolol and additional drugs Drug therapy has failed in 7 patients and in 6 of them a trabeculectomy was performed (patients Nos 6 12 22 23 30 and 31) Three of these had [illegible] tension levels in the fellow non-operated eye using drug therapy as illustrated in Fig 2 (patients Nos 6 22 and 31)

Table II shows the comparison between the therapeutic response to the previous and to the present therapy Out of 18 patients who could be well regulated on Timolol alone 13 had previously used two local drugs and another 4 had [illegible]

Table II
Comparison of prior tharapy and present medication.

PRIOR THERAPY	NO PAT	PRESENT THERAPY NO PAT WITH I O P < 22 mm Hg TIMOLOL	TIMOLOL EPINEPHR	TIMOLOL PILO	LOCAL DRUGS ACETA OL.	[illegible]
NONE NEW CASES	2	1			1	
ONE LOCAL DRUG (PILO 7 EPI 1)	8	5	1			
TWO LOCAL DRUGS (PILO EPI)	9	8	1			
LOCAL DRUGS						
ACETAZOLAMIDE	17	4	1	1	4	
ACETAZOLAMIDE	1				1	1
TOTAL	37	18	3	1	6	
I O P > 22 mm Hg ON PRIOR THERAPY AMONG THE 37 PATIENTS	16	6	2	0	2	1

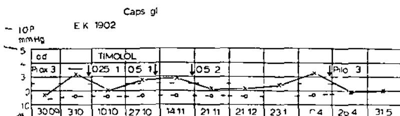


Fig 3

ire curve from a patient showing a relative slowly developing insensitivity to Timolol reverting to normal level when converted to pilocarpine

ed acetazolamide Among the 18 patients who had previously used acetazo-le six had no need for this drug while on the present therapy On prior py 34 patients had to use piolocarpine 3 times a day and/or acetazolamide of these patients were well regulated on Timolol alone with application one (14 nts) or two (3 patients) times a day Among the 9 patients with iop above 22 Hg drug treatment failed on one eye in 4 patients while the glaucoma in the eye was well regulated

it of 16 patients who with previous medication had iop above 22 mm Hg 10 nts were well regulated by the new treatment and 6 of these with Timolol The average reduction of iop in the total group was about 23% with standard ation 16 4

ie effect of Timolol maleate could be registered already 9 respectively 15 h the first application Some patients however subsequently developed relative isitivity to the drug requiring a stronger solution of Timolol or the addition of entional drugs Such relative insensitivity was already oberved in one patient ng the first week of treatment and in another case during the first 5 to 6 iths (Fig 3)

onography has shown almost no change in the facility of outflow not even when op dropped from 46 to 16 mm Hg from one day to the next

effect of Timolol on different types of glaucoma

Figs 1 & 2 it is shown that only one of the 9 patients with capsular glaucoma ld be maintained on Timolol as the sole drug while 17 of the 26 patients with ple glaucoma required Timolol medication alone Surgical treatment had to be formed in 4 patients with capsular glaucoma (patients Nos 6 23 30 and 31) le trabeculectomy was performed in only 2 patients with simple glaucoma tients Nos 12 & 22)

The average reduction of the iop on medical treatment was approximately [illegible] in 11 eyes with capsular glaucoma and approximately 26% in 48 eyes [illegible] glaucoma

The two patients with chronic secondary glaucoma (patients Nos. 9 & 33 [illegible] satisfactory pressure levels on the new regimen and one of these required [illegible] Timolol to maintain this Both these patients had previously needed [illegible] treatment and one of them was even then not adequately controlled.

Side effects Two of the patients showed at one visit a pulse rate below 60 per [illegible] but had no subjective symptoms and the frequency was easily increased by [illegible] excercise No influence on blood pressure was recorded During treatment [illegible] Timolol as the only drug the pupillary diameter has been about the same as [illegible] during the wash-out period and the pupillary reactions have been normal [illegible] have seen no changes in the conjunctiva cornea, lens or retina No progressive optic nerve damage have been observed We have not recorded any lowering of [illegible] tear production is judged by the Schirmer test

Discussion

This study supports the earlier reports that Timolol maleate has a marked pressure reducing effect when applied locally to open angle glaucoma The mode of action seems to be a lowering of aqueous humour production with no alteration of the outflow facility This agrees with earlier observations (Zimmerman et al. 1977) The present study is an open study not randomized and comprises a relatively [illegible] proportion of glaucomas difficult to treat Thus 14 patients have been ad[illegible] the department and 11 patients had glaucoma of such a severity that [illegible] considered the only alternative It is worth noting that 5 of these [illegible] satisfactorily regulated on the new medical therapy while the remaining 6 had to undergo surgical treatment Among the 37 patients 18 were maintained satisfactorily on Timolol alone while the remainder needed the addition of additional drugs (13 patients) or surgery (6 patients)

The effect of Timolol is seen by a decrease of the iop the day after the first application The relative insensitivity to the drug seen in some patients is [illegible] explained by an initial dramatic drop of aqueous production with a later stabilization at a higher level

Our study indicates that capsular glaucoma responds less well to Timolol than the simple glaucomas as 65% of the patients with simple glaucoma required Timolol as the only form of treatment compared to 11% of the capsular glaucomas

gst the *16 patients with iop exceeding 22 mm Hg on previous treatment 3* ipsular glaucoma and all these had iop exceeding 22 mm Hg at least in one so on present medication This confirms the earlier observation that capsular oma is a more difficult type of glaucoma to treat than simple glaucoma ed 1971 Kertu & Horven 1978)

plied locally Timolol maleate has a prolonged effect Out of the 18 patients ained on Timolol as the only drug 15 needed only one application a day As terval between application and iop registration was 15 h for the outpatients p to 23 h for the inpatients this study supports earlier investigations that the of Timolol lasts longer than 24 h (Zimmerman et al 1977)

is study also confirms the absence of side-effects using this drug This has been aneously expressed by many patients who enjoy better sight and night vision miosis of earlier treatment have disappeared The reduction of applications 2 times a day must also be of great benefit

this investigation the pressure reducing effect of Timolol in most cases has *almost unchanged during the period of observation If the pressure reducing* t of Timolol maleate proves to be a lasting one over several years of treatment out any serious side effects this might well represent the beginning of a new era treatment of open angle glaucoma

ir present experience indicates that Timolol maleate in many instances will be rug of choice

Acknowledgment

l lmaleate (Blocadren®) used in the present study was kindly provided by Merck Sharp ohme

References

ed H (1971) The frequency of optic nerve damage and surgical treatment in chronic ngle glaucoma and capsular glaucoma *Acta phthal (Kbh) 49* 589–600

ami L & Steindler P (1975) Effect of pindonol on intraocular pressure *Brit J Ophthal* 301–303

hne A (19) The treatment of glaucoma with propranolol (Inderal®) A clinical trial *ta phth l (Kbh) 54* 291–300

ikman R F (1976) Ocular hypotensive effect of atenolol 4% eye drops in glaucoma In um Ophthal Proc Ser vol 1? pp 85–94 Cree E I ed Su p ... n d al ... a A n ter lam 19 n D W Junk the Hague 197

C & Drance S (1968) The effect of propranolol on human intraocular pressure *Canad Ophth l 3* 9?? –919

Katz I M Hubbard W A Getson A J & Gould A L (19) Intraocular pressure decrease in normal volunteers following Timolol ophthalmic solutions Invest Ophthal ...
Kerty E & Horven I (1978) Glaucoma treatment with Timolol Acta ophthal (Kbh) ...
70 –714
Kriegelstein G K Sold Darseff J & Leydhecker W (19) The intraocular pressure response of glaucomatous eyes to topical applied bupranolol Albrecht v Graefes Arch klin Exp Ophthal 202 81–86
Musini A Fabbri B Bergamaschi M & Mandelli V (1971) Comparison of the effect of propranolol lignocaine and other drugs on normal and raised intraocular pressure in man Amer J Ophthal 72 773–781
Nielsen N V (1978) Timolol Hypotensive effect used alone and in combination for treatment of increased intraocular pressure Acta ophthal (Kbh) 56 504–509
Phillips C I Howitt C & Rowlands D J (196) Propranolol as ocular hypotensive agent Brit J Ophthal 51 222–226
Phillips C I Gore S M McDonald M J & Cullen I M (19) Atenolol eye drops in glaucoma A double masked controlled study Brit J Ophthal 61 349–353
Stuhr J S (1976) Use of oxprenolol eye drops in glaucoma patients In Docum Ophthal Proc Ser vol 12 pp 9 –97 Greve E L ed Symposium on medical therapy in glaucoma Amsterdam 1976 Dr W Junk the Hague 1977
Vale J Gibbs A C C & Phillips C I (1972) Topical propranolol & ocular tension in the human Brit J Ophthal 56 770–775
Vareilles P Silverstone D Plazonnet B LeDouarec Sears M L & Stone C A (19) Comparison of the effects of Timolol and adrenergic agents on intraocular pressure in rabbit Invest Ophthal & Visual Sci 16 987–996
Wettrell K & Landolfi M (1975) Effect of oral administration of various beta blocking agents on the intraocular pressure in healthy volunteers Exp Eye Res 21 451–456
Wettrell K & Landolfi M (1977) Effect of topical atenolol on intraocular pressure Brit J Ophthal 61 334–338
Zimmermann T J & Kaufmann H E (1977a) Timolol Beta adrenergic blocking agent for the treatment of glaucoma Arch Ophthal 95 601–604
Zimmermann T J & Kaufmann H E (1977b) Timolol Dose response and duration of action Arch Ophthal 95 605–607
Zimmermann T J Harbin R Pett M & Kaufmann H E (1977) Timolol and facility of outflow Invest Ophthal & Visual Sci 95 623–624

Author s address
Department of Ophthalmology
N 5016 Haukeland sykehus Bergen Norway

Department of Ophthalmology (Head R T rnquist)
Region Hospital Oreb o Sueden

CAVERNOUS HAEMANGIOMA OF THE RETINA

BY

INGIMUNDUR GISLASON STAFFAN STENKULA ALBERT ALM
EIVIND WOLD and PER ERIK WÅLINDER

Seven cases of retinal cavernous haemangioma are presented Three cases have been followed for more than 6 years and three cases between 1 and 2 years Six cases had no eye symptoms related to the vascular tumour while in one case vitreous haemorrhage occurred on two occasions On both these occasions full vision was regained None of the vascular tumours were treated Two patients had grand mal seizures They also had convulsive disease in the family history In three cases family members of two generations were found to have normal eyes on examination

Key words Cavernous haemangioma — retina — vascular disturbances — epilepsy

mous haemangioma of the retina and optic disc is a specific vascular artoma It is supposed to be inherited in an autosomal dominant mode and be associated with similar lesions of the skin brain and other organs (Gass) The tumour consists of a group of round dark red aneurysms sometimes dded in white fibrous like tissue Fluorescein angiography demonstrates ed perfusion and a characteristic appearance of plasma erythrocytic layering n the aneurysms Leakage of the dye does not occur Haemorrhages from this lar malformation are rare and exsudation has never been reported

wis et al (1975) published three cases and presented a review of 35 cases

of this work was presented to the Swedish Ophthalmological Society in Stockholm mber 29 to December 2 1978
ved February 19 1979

previously reported in the literature Klein et al (1975) reported 4 [illegible] and Winning (1976) presented one case each Patton et al (1976) showed two cases in their atlas Colward et al (1978) reported 2 cases associated with [illegible] features

In this report we describe 7 cases examined in Sweden during the period [illegible] 1978

Case Reports

Case 1

Female born in 1940 The patient had suffered from intermittent pains in the left side of [illegible] face for many years but was otherwise in good health A 16-year-old sister died from a [illegible] haemothorax of unknown cause The patient had a severe vitreous haemorrhage in [illegible]



Fig 1

Case 1 Large aneurysms in the periphery of the right fundus Erythrocytic sediment [illegible] the upper aneurysm

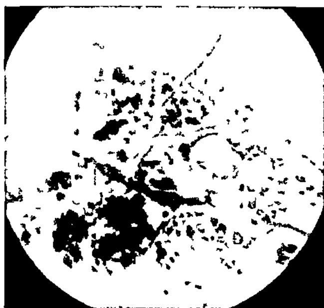


Fig 2
2 A nasal sector of the left fundus Aneurysms of different sizes Some of the largest appear to be fibrotic

r bleeding in 1976 On both occasions she regained full vision After the first bleeding evated fibrous mass with cystic thin walled formations was found in the temporal hery of the fundus Several large cysts embedded in the mass were filled with dark l, which showed the typical sedimentation of large cavernous haemangiomas (Fig 1) other eye was normal There seemed to be no change in the lesion at examinations up to

2

born in 1939 an immigrant from Yugoslavia The family history was unremarkable as in good health and had never had any eye symptoms When he was treated for a or corneal injury in 1966 a retinal vascular malformation was found in the periphery of eft eye (Fig 2) It consisted of round grape like aneurysms filled with blood in a grey minent mass of tissue Otherwise the eyes were normal The patient was not available for her examinations

3

born in 1943 His father had epilepsy and a son was mentally retarded and had epsy In 1965 a vascular skin tumour was removed from his leg Pathological examination ved a haemangioma with traumatic ulcerations In 1969 he had an ophthalmological mination after two incidents of grand mal convulsions His vision was normal and he had ye symptoms In both fundi clusters of saccular aneurysms were found in the periphery 3) Otherwise the eyes were normal Pneumoencephalography and cerebral angio-

graphy were normal Subsequent examinations have not revealed any changes in the
Fluorescein angiography in 1977 showed the typical picture of cavernous haema
slow perfusion and plasma erythrocytic sedimentation The patient s son was not
eye examination

Case 4

Male born in 1956 The family history was negative In 1979 he was found to have
vision with myopic correction In the periphery of the right fundus there were
formations The tumour was encircled by a small rim of fibrous like tissue
normal He had no history of vitreous bleedings or convulsive disease
reexamined in 1978 When photographs from this examination were com
from 1972 it was found that some of the aneurysms had been replaced by fi

Case 5

Male born in 1907 His medical history includes bronchial asthma, heart
an operation for a thyroid adenoma He had one attack of grand mal in 1
in 1977 There was no family history of ocular diseases An uncle had convuls
a son had epileptiform attacks thought to be caused by a previous slight
Ophthalmological examination after the grand mal attacks in 1977 revealed
dilated vascular aneurysms in the retina of the left eye The visual acuity
Fluorescein angiography showed a slow perfusion and plasma erythrocytic layer
phase (Fig 4) Neurological examination including computed tomography was normal
had no vascular skin tumours Four of the patients children including the son
convulsive disease were examined and they were found to have normal eyes

Case 6

Male born in 1966 He was in good health The family history was negative In 1 he
examined for astigmatism The corrected visual acuity was 0 4 in the right eye and 1 0 in the
left eye In the right fundus there was a vascular wedge shaped tumour in the periphery
consisted of a protruding dark blue mass of dilated vessels filled with blood Fluorescein
angiography confirmed the diagnosis of cavernous haemangioma (Fig 5) The left eye was
normal On reexamination in 1978 the eye tumour was unchanged His parents, a sister and
brother were examined and had no eye diseases

Case 7

Female born in 1973 The family history was negative On routine examination in 197
large vascular tumour was discovered in the nasal peripheral part of the right fund (Fig 6
The tumour was built of round dark blue saccules some of them were surrounded by wh
or grey tissue Fine retinal folds extended from the tumour to optic disc Other parts of the
fundus and the other eye were normal She had full vision and had never had an ey
symptoms Neurological and paediatric examination were normal No haemangiomas were
found in the skin Reexamination in October 1978 showed no changes of the lesi
Her parents and only sister were found to have normal eyes

Fig 3

Case 3 Right eye with a cavernous haemangioma Lattice degeneration in the periphery

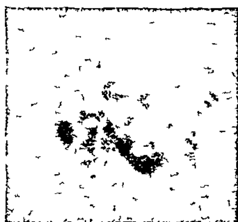


Fig 4

5 Left eye Fluorescein angiogram Erythrocytic sedimentation in the late phase of the fluorescein angiogram



Fig. 5

ase 6 Fluorescein angiogram right eye Arterio-venous phase Notice erythroc ... and delayed drainage in one of the veins (arrows)



Fig 6

e 7 Right fundus A big vascular tumour in the nasal periphery Stretching of vessels towards the lesion

Discussion

of the patients in our material have been followed during periods of different ngth from 1 to 22 years We have not found any progress of the lesions in any of ese cases Krause (1971) reported a case which had been observed for 13 years d showed slight progress Lewis et al (1975) reported no progress in a case ved during 34 years In one of our patients the lesion showed increased brotic changes (case 4)

These tumours seldom give symptoms One of our cases had two episodes of itreous haemorrhages but regained full vision Three patients have previously een reported to have bilateral lesions (Scheyhing 1937 Reese 1963 Frenkel & usse 1967) One of our patients had retinal haemangiomas in both eyes (case 3) It



Fig 5

Case 6 Fluorescein angiogram right eye Arterio venous phase Notice erythrocyte [illegible] and delayed drainage in one of the veins (arrows)

ı D Hyman B N & Justice J (1976) *Ii troduction to ophthalı ıo copı* p 60 Upjohn npany Michigan
ㄅ B (1963) *Tumors cf tl e eye* 2nd ed p 380 Harper and Row Publishers New York
hing H (193/) Ein seltener Fall von angiomatosen Veranderungen der Netzhaut *Kl* ' *Augenheilk.* 99 36?–367
ıng C H O M von (19 6) Haemangiomas of the fundus *Docum ophthal* 40 2 –381

rs ad lress
ıundur Cı lsson M D Department of Ophthalmol gy Regı n Ho pıtal S 01 8 ro Sweden

Eye Department (Heads I Brænd trup S F Lorentzen M S Norn and K Ne) Copenhagen City Hospital and Eye Department (Head F Wewalsh) Central Hospital Nykøbing Falster Denmark

THE PALLOR OF THE OPTIC DISC

A quantitative photographic assessment by purple li

BY

PER NELLEMANN SORENSEN

By employing purple colour filters with a large blue and a small transmission normal and atrophic optic disks were photographed on c transparencies Normal optic disks reflect red light and atrophic reflect blue light The degree of atrophy estimated by colour quantitated by microdensitometry in the blue (4 0 nm) and in the red region of the transparencies The difference between the blue and red is used as an expression of optic disc colour

Key words microdensitometry – optic atrophy – colour contrast – interfer filter – *purple filter* – photography – quantitative measurement optic ophthalmoscopy

The degree of pallor of the optic disc is considered a means of evaluating atrophy The colour of the normal disc is due to vascularization since tissue is grey (Walsh & Hoyt 1969) When the optic nerve fiber bundles a white reflecting glial tissue is formed on the optic disc the vascularization decreases ophthalmoscopically and a corresponding pallor of the disc is se (Quigley & Anderson 1977)

The purpose of this paper is to introduce a qualitative or quantitative photographic method of assessment of the pallor of the optic disc by means of a nd interference filter in the light path The filter transmits a large amount blue light and a small amount of red light The blue light is mostly absorbed by the haemoglobin in the vessels and scattered to a lesser degree whereas the red light is primarily reflected from the haemoglobin giving rise to the red impression of

Received November 8 1978

– ization often on a blue contrasting background Since the overall trans 1 of the filter is blue the colour impression of the disc is blue when the disc is almoscopically avascular On the other hand when the vascularization is only decreased a mixture of red and blue is seen and this purple colour sion is easier to define than shades of red alone

his method allowance is made for variations in exposure and processing colour photography is performed

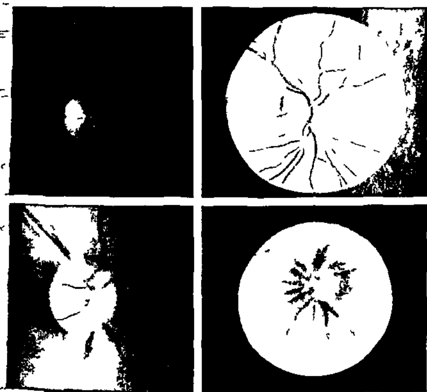


Fig 1–4

1 Temporal pallor of the optic disc due to multiple sclerosis photographed with purple

blue colour denotes atrophy and red normal tissue (Flash 350 Wsec Kodachrome 25)

2 Temporal pallor of the optic disc photographed without filter (the same patient as in 1 Flash 180 Wsec Kodachrome 25) × 5

3 A normal optic disc photographed with purple filter The red optic disc contrasts with bluish retina. × 10

4 A glaucomatous optic disc photographed with purple filter The atrophy is seen as a ish colour × 5

Methods

Photographs are taken with a standard Zeiss (Oberkochen) fundus camera. The f[illegible] is used is of a multilayer interference type which is used for colour fluorescein a[illegible] and described by Jensen & Olsen (1974). This filter has a 85 per cent blue transmission fr[illegible] 425–505 nm and a narrow red band at 640 nm with a half band width of 8 nm, transmi[illegible] 50 per cent.

Kodak wratten 32 (Magenta) alone or in combination with wratten 59 which a c[illegible] in the Kowa and Topcon fundus cameras were also tested but the red transmissio[illegible] to be too high.

Pictures were taken with the pupils dilated with colour diapositive films of subtractive [illegible] colour coupled (Agfachrome professional 50 S (5500 K) or Ektachrome X and Fuji [illegible] which had their colour laid on during the development (Kodachrome 25 and 64).

Pictures were taken with and without filters at varied exposures by variation of the [illegible] flash intensity which was regulated via the charging voltage.

The Ektachrome films were developed by a private laboratory by process E-4 the [illegible] films by the manufacturers.

A variation due to change in the light sensitive material by time or manufacture [illegible] variation due to change in development was checked by photographing a minimized [illegible] paper copy of the star shaped Kodak enlarging negative on each film.

According to information provided by the manufacturers there is only a small overlap [illegible] of sensitivity between the cyan and yellow forming layers of the films which makes [illegible] respectively blue colours on the final transparency. A sensitometric curve was constructed [illegible] densitometry employing a microdensitometer consisting of a Reichert microscope [illegible] built in Schott Verlauffilter with 15 nm half band width. The density was read at 4[illegible]0 [illegible] 640 nm corresponding to the maximal transmittance of the blue and red colour. [illegible] resulting curve was linear with a correlation coefficient $R^2 > 0.95$ for both blue and red. [illegible] slope varied less than 5 per cent between blue and red in agreement with the sensi[illegible] data from the manufacturers (from Fuji no data were obtained).

On the optic disk pictures the density was read on the vascular rim below the s[illegible] temporal vessels the measuring area correspond to 50 μ in the emmetropic subject. T[illegible] difference between the densities at 470 and 640 nm $D_{470}-D_{640}$ was taken as an arb[illegible] index of the colour balance between blue and red. Thus variations in the exposure [illegible] processing of the subtractive films affects the densities in the same direction.

Results

Examples of the blue and red filter technique are seen in Figs 1 3 & 4.

Figs 1 & 2 demonstrate the difference between a fundus photograph of a patie[illegible] with temporal pallor of the optic disc in white light (120 W/sec Kodachrome [illegible]) and with the blue/red filter (350 W/sec). The patient is a 21 year-old woman with multiple sclerosis which has caused a central scotoma with vision decreased to 6/1[illegible]

Table I

ifference between the den ities at 470 nm and 640 nm on the vascular rim of optic disc filter photographs Five films are compared in two patients with optic atrophy in one d a normal fellow eye The mean density of two photographs in each eye is given

	Film Speed (ASA)	Flash W sec	Optic disc D_{470}–D_{640}			
			Normal		Atroph c	
			I	II	I	II
hrome 50S	65	240	0 92	1 02	0 62	0 72
hrome X	65	240	0 84	0 76	0 62	0 58
chrome 25	25	350	1 52	1 62	0 52	0 05
chrome 65	65	240	2 35	1 94	1 05	1 90
	100	180	0 98	0 84	0 68	0 [illegible]2

ble I demonstrates that Kodachrome 25 film gave the greatest difference een a red vascularized area and a pale atrophic area of the optic disc The ur balance of the optic photographs the amount of blue and red is expressed he difference between the densities at 470 and 640 nm Thus a small value otes a bluish hue and a high value a reddish hue

able II demonstrates that only at flash intensities above 240 W sec the D_{470}–D_{640} onstant and a linearity exists between film density and exposure for blue and light Below 240 W sec the density at 640 nm exceeded 3 0 and thus the proper osure is passed as found by the exposure card

surements on transparencies with the optic disc pictured in the periphery or harp pictures gave a bluish false balance

Table II

blue and red colour balance of normal (n = 3) and atrophic (n = 3) optic discs at aried osures on Kodachrome 25 transparencies and with purple filter

Flash intensity (W sec)		120	180	240	350
ic disc D_{470}–D_{640}	Normal	0 05	0 72	1 40	1 45
	Atrophic	–0 15	–0 28	–0 31	–0 32

Discussion

With the described purple (blue/red) filter technique for estimation of the colour of the optic disc difficulties lie in the control of exposure i e the illumination of the fundus of the eye which may vary with changes in the optical media and the type of illumination due to small eye movements and variation in film and development. With the filter technique changes in the spectral light distribution due to changes of the glow lamp intensity or type of lamp are decreased and they can be avoided by means of bluish conversion filters

Changes in the optical media are usually due to increased light absorption by the ageing lens of the patient and observer (Miller & Benedek 1973) Since an apparent increase in red colour of more than 30 per cent between the ages 0-65 [illegible] (Said & Weale 1959 Boettner & Wolter 1962) a correction for the age group is necessary The change due to variations in exposure is accounted for by the use of two measurements at different wavelengths at only one spot on the optic disc

An error due to different sensitivity of the blue and red sensing [illegible] is minimal as seen from the sensitometric curves of the blue and red sensing [illegible]. Furthermore the error is multiplicative and not additive

Schwartz (1976) has solved the problem of variation due to changes of [illegible] intensity and film development by photographing a step wedge simultaneously with the fundus However this error appears to be small compared to changes in the optical media (Sørensen 1977)

The measurement of colour contrast for estimating the degree of pallor of the optic disc has also been studied by Bock (1950) Gloster (1969) Berkowitz & [illegible] (1970) Davies (1970) and Gloster & Parry (1974)

By using polychromatic film we have adopted the ideas of Gloster (19[illegible]) [illegible] Davies (1970) who measured the green and red density of white light [illegible] parencies for obtaining a measure of vascularity from the reflection of haem oglobin But we have also incorporated the old idea of using red free [illegible] (Gullstrand 1906 Vogt 1918) by using a filter with a dominantly blue transmission

Thus these and our methods rest on the assumption that pallor of the optic disc is synonymous with avascularity but pallor may represent tissue in which rearrangemant of astrocytes in dense parallel layers has taken place resulting in altered light scattering and reflecting (Quigley & Anderson 19[illegible]). [illegible] fluorescence of the optic disc in fluorescein angiography in normals does [illegible] correlate with the ophthalmoscopic observations (Hayreh 1969 19[illegible]). However when optic atrophy is present (O Day et al 1967 Hayreh 1969) the fluorescence [illegible] generally reduced Also with patent blue V deep red absorption angiography [illegible] 640 nm the optic disc is even filled in normals (Sørensen 1977)

Gloster & Parry (1974) in their cyan filter methods for estimation of [illegible]

matous cupping and pallor point to the possibility that at least a part of the ed light from the optic disc comes from light diffused into the optic disc rim the choroid This problem is of greater importance in evaluating the omatous optic disc with a narrow vascular rim but slit lamp examination ying contact lens shows that the optic disc generally first begins to glow ntly when the slit reaches the optic disc border and then the entire disc s due to its translucency (own observation)

in all the described purple filter with its potential of quantitative technique be an improvement on the common red free filter technique for the ation of optic disc pallor by colour contrast

Acknowledgments

sen M Sc Optisk Laboratorium DK–2800 Lyngby is thanked for obtaining the purple aver interference filter

rchant A V Lykfeldt and wife s Foundation is thanked for economical support

References

witz J S & Balter S (1970) Colorimetric measurement of the optic disc *Am r J opl thal* 385–386

R H (1950) Zur klinischen Messung der Papillenfarbe *Ophthal nologica 120* 174–177

ner E A & Wolter J R (1962) Transmission of the ocular media *I n e t Ophthal 1* – 83

y D Crock G Galbraith J E K Parel J M & Wigley A (1967) Fluorescein giography of normal and atrophic optic discs *Lanc t 2* 924–926

es E W G (19 0) Quantitative assessment of colour of the optic disc by a photographic thod *Exp Eye Res 9* 106–113

ter J (1969) The colour of the optic disc *Doc Opl th l 26* 153–163

ter J & Parry D G (1974) Use of photographs for measuring cupping in the optic disc *J Ophthal 58* 850–862

trand A (1906) Die Farbe der Macula central s retinae *on Graefes Arch Ophth l 62* 2

reh S S (1969) Blood supply to the optic nerve head and its role in optic atrophy aucoma and oedema of the optic disc *Brit J Ophthal 53* 721–748

reh S S (1972) Colour and fluorescense of the optic disc *Ophthalmol gica 165* 100–108

sen V A & Olsen W (1974) Colour fluorescein angiography of the fundus *Acta opl tl al* (Kbh) 52 501–511

ler D & Benedek G (1973) *Intraocula light tteri g* p 29 Ch C Thomas Publ pringfield

igley H A & Anderson D R (1977) The histologic basis of optic disk pallor in xperimental optic atrophy *Amer J Ophthal 83* 09–717

Said F S & Weale R A (1959) The variation with age of the spectral transmission of the living human crystalline lens *Gerontologica 3* 213–231

Schwartz B (1976) New technique for the examination of the optic disk and its clinical application *Trans Amer Acad Ophthal Otolaryng 81* 227–237

Sørensen P N (1977) Choroidal absorption angiography with patent blue V [illegible] *(Kbh) 55* 471–477

Vogt A (1918) Ein ophthalmoskopisches Symptom der Netzhautatrophie [illegible] Netzhaut und Sehnervenerkrankungen in besonders von Neuritis retrobulbaris [illegible] *Mbl Augenheilk 60* 324–329

Walsh F B & Hoyt W F (1969) *Clinical Neuro Ophthalmology* 3rd ed p 6[illegible] W[illegible] Wilkins Baltimore

Author's address

Per Nellemann Sørensen M D Eye Department 2061 Rigshospitalet
DK 2100 Copenhagen Ø Denmark

Health Service of the Hungarian People s Army Hospital No 2
Department of Ophthalmology (Head I Tapaszto) and
Novex Foreign Trade Co Ltd. for Development and Commercialization of Inventions
Budapest Hungary (Head J Salusinszki)

BIOPLAST® FIBRIN FILM FOR CONJUNCTIVAL REPLACEMENT

BY

ISTVAN TAPASZTO and GEZA KERENYI

The study includes 43 cases of conjunctival grafting in chemical burns and traumatic pterygium Resorbable Bioplast® fibrin film was used as a readily available biocompatible conjunctival substitute The implant was absorbed and the site occupied by fresh conjunctival tissue in a few weeks The composition of tear proteins was restored to normal as fast as after free conjunctival grafting The results were also satisfactory in terms of cosmetics

Key words Bioplast® – fibrin – conjunctival replacement – biocompatibility

...strialization has led to an increasing occurrence of eye injuries Severe acid and ...rns may necrose large areas of the conjunctiva and result in corneal opacity ...storting scars

...r the immediate chemotherapeutic measures the treatment of these injuries ...ed on an operation for replacing the necrosed conjunctiva. Literature is ...dant in reports on the use of various conjunctival grafts The materials tested ...le canine conjunctiva (Wolfe 1873) human conjunctiva (De Wecker 1879) ...l and vaginal mucosa (Stellwag von Carion 1889) amnion (de Totth 1940) ...neum (Malhotra 1957) prepuce (Soliman 1969) silicone rubber (Merz & ...inska 1969) etc The variety of these materials shows that none is sufficiently ...le to find general acceptance although human conjunctiva (Gundersen flap) ...uccal mucosa are quite suitable in many situations The major disadvantages ...njunctival substitutes are limited availability difficulty of storage postoperative ...trophy unsatisfactory cosmetics etc

...ved February 22 19 7

A conjunctival autograft is certainly the best solution but its use is not feasible in injuries extending over large areas of one or both eyes. In most cases, buccal mucosal grafts are applied as a substitute for exercised tissue.

Our objective was to test Bioplast fibrin films for this purpose and to see whether the method compares favourably to currently appl ed techniques.

Material

Patient material

The study comprises a series of 43 cases of conjunctival grafting with Bioplast in 32 patients (25 males 7 females) Of the 43 injuries 11 were fresh chemical 12 burns occured ealier and resulted in scar formation and symblepharon and there were 20 cases of traumatic pterygium Most injuries were caused by lime or the lesions studied over a period of 4 years were too extensive to be solved by grafting with autogenous conjunctiva

A control group included 6 alkali burnt patients (4 males, 2 females) treated with free transplantation of conjunctiva from the other eye and burnt patients (6 males 2 females 10 eyes) grafted with oral mucous membrane

The follow up time amounted to at least 4 years after the latest operation in the experimental series and in the control group

Implant material

Bioplast is a biocompatible absorbable implant material made from subcured bovine fibrin Endoprostheses are prepared by compression moulding a mixture of powder glycerine and water (Gerendas 1970)

The resorption time of the implants can be modified by crossorlinking with formaldehyde (Gerendas 1968)

No infection fever or undue histological reaction follows implantation (Gerendas No clinical symptoms of an immunological response can be detected even after the implantation of the heat deantigenized bovine material (Horvath et al 1975)

Resorption proceeds at an even rate and metabolites are eliminated while the site is gradually invaded by connective tissue (Torok et al 1975)

Buttons were applied for the protection of liver sutures (Wood et al 1961) and reported as useful aids for scleral buckling in retinal detachment (Crost et al 1974) been successfully applying films for skin replacement in ophthalmology (Tapaszto et al 1977) Bean shaped implants were used in female urinary stress incontinence 1975)

Other future applications of Bioplast implants have been suggested (Gerendas 1977)

The first industrially produced Bioplast implants were put on the market by Ebhauser in autumn 1976 They are sold under the registered name Biethium.

E.. Bioplast films used for conjunctival grafting were prepared from human fibrin They
ar 0×90 or 20×20 mm in size and 0 2 mm in thickness They were soft and easy to
due to their high glycerine content. They were moderately crosslinked and had a
tion time of 2–3 weeks The sterile Bioplast films were obtained as materials for clinical

Method

ique

ry for recent injuries is scheduled as soon as possible since these cases are
,encies

tillation of adrenaline (Suprarenin Epinephrine) and cocaine is followed by
ccision of the affected conjuctiva in local anesthesia (0 001% adrenaline + 2%
dcaine (Xylocaine injection) in the case of fresh injuries In older injuries with
ing scarred conjuctiva is excised and when a symblepharon is present
sions of the bulb and eyelids are separated After excising the necrosed tissue
i to normal tissue where bleeding occurs conjuctival replacement is performed
the thin Bioplast fibrin film of which a piece is cut corresponding to the size of
nissing conjuctiva (no contraction of the implant was observed) The film is
ed in place with 7–0 silk black braided Ethicon sutures It is anchored to
hy conjuctival edges so that the margin of the graft is covered by the host
An antibiotic ointment (Terramycin and Niomycin) is then placed in the
ative area and the eye is bandaged The dressings are changed twice a day
res are removed 6 days postoperatively
ical antibiotic administration is discontinued 10 days postoperatively

hemical methods

ddition to studying the clinical efficiency of Bioplast fibrin grafts a comparison
he biocompatibility of free conjuctival and buccal mucosal transplants and
plast was performed Postoperative tear protein content and composition were
rmined to this end The methods were reported elsewhere (Tapaszto 1979)

Results

iical results

e clinical course was reassuring in all 43 cases A good take of the graft was
ays seen Pronounced exudation was seen on the first few days following
plantation The implant was initially transparent and yellowish then became
white exhibiting the fibrin network No more discharge was seen after suture
noval 6 days *postoperatively At this time Bioplast still appeared as a whitish*
stance but the beginning of vascularization was already observed along the

edges No shrinkage of the implant was seen (Fig 1) Fifteen days after implantation the Bioplast film had been transformed into or replaced by conjunctiva The cornea was covered by fresh tissue which did not differ from the surrounding cornea (Fig 2)

Of the 11 fresh chemical burns slight symblepharon formation was noted cases at the follow up 1 year postoperatively They were definitively resolved by repeated Bioplast implantation Of the scar and symblepharon cases of earlier burns 3 symblepharons needed reoperation while of the 90 cases of traumatic pterygium 5 had to be reoperated once

Fig 1

Traumatic pterygium on the right eye 7 days after Bioplast implantation



Fig 2
The eye shown in Fig 1 15 days postoperatively

In the control group of 6 cases treated with conjuctival transplantation from the other eye 2 had to be reoperated. Of the 8 control cases where oral mucosa was transplanted 3 cases required one reoperation.

The situation achieved by the original intervention of the reoperation when repeated surgery was needed remained unchanged until 4 years after the last operation which was the follow up time in all cases.

Biochemical results

When no operation is performed normal tear total protein content (approximately

800 mg/100 ml) is restored about 20 days after an alkali burn Normal values of tear total protein and normal protein fraction composition are restored about [illegible] after the free transplantation of conjunctiva from the other eye [illegible] days after grafting with buccal mucous membrane and 8 days after implanting Bioplast film (Tapaszto 1973)

Discussion

Recording the clinical course total tear protein tear protein fraction composition and the cosmetic results showed the Bioplast fibrin graft to be a favourable conjunctival substitute It is equivalent to autogenous conjunctiva and can be recommended in severe chemical burns highly limiting the availability of healthy tissue The fibrin implant irritates the eye to the least degree is replaced by conjunctiva as it acts almost as a template for the proliferation of fresh tissue and neovascularization from the episclera and surrounding healthy conjunctiva New cascular conjunctival tissue is found at the site 2–3 weeks postoperatively

Suppuration is readily prevented by the postoperative treatment No rejection occurs and the cosmetic results are good after several years The time of post-operative healing is not longer than after free conjunctival transplantation and is shorter than after implanting other materials The Bioplast implant is transformed into functional conjunctiva

In traumatic pterygium Bioplast is indicated only if there is a good prospect that the corneal epithelium can be restored before the implant is degraded and new tissue develops

With older scarred injuries and symblepharon the implanted Bioplast film can be in contact with the wound surface on one side only The other side of the implant should lay free or if it is in fornices the conjunctiva must be healthy on the other side of the fornix

Bioplast was found particularly helpful in older injuries causing the formation of distorting scars thus reducing the amount of healthy conjunctiva When the scarred tissue is excised large areas can be covered with the graft, stimulating the formation of new conjunctiva and yielding good cosmetic results

Acknowledgment

The authors wish to thank Novex Co Ltd Budapest, for supplying the implant material and giving valuable technical assistance in documentation

References

Capperauld I Lawrie P & French D A (1977) Properties of bovine fibrin absorbable implants Surg Gynec Obstet 144 3–7

Ferendas M (1968) In Laki K ed Fibrinogen Chapter 13 Marcel Dekker Inc New York

Ferendas M (1976) Improvements in or relating to prostheses U S Patent No 3 523 807

Gosz I Vereb K & Kerenyi G (1976) Scleral buckling with Bioplast® fibrin in retinal detachment Acta ophthal (Kbh) 54 408–416

Gorin B Kover J & Marton I (1975) Treatment of stress incontinence by a fibrin Bioplast. Brit J Obst Gynaec 82 61–63

Horvath N Surjan J & Somogyvari K. (1975) Studies on antibody response after implantation of fibrin Bioplast Ann clin res 7 54–58

Malhotra M (1975) Plastic repair of conjunctiva with peritoneum transplantation Brit J Ophthal. 41 616–6?1

Mertz M & Czerwinska W (1969) Silicone rubber in defects of the conjunctiva Brit J Ophthal 53 46–48

Totth A (1940) Plastic repair of conjunctival defects with fetal membranes Arch Ophthal. (Chicago) 23 522–525

Soliman A M (1952) Conjunctivoplasty Bull Soc Ophthal Egypt 45 97–99

Stellwag von Carion K (1889) Ruckblicke auf die augenartzlichen Pfropfungsversuche und ein neuer Fall von Schleimhautubertragung Allg Wien med Ztg 34 305–306

Straub W (1969) Uber die Verwendung von homologem Bindehautgewebe zur Deckung von Konjunktivaldefekten Kl n Mbl Augenheilk 155 258–259

Szapaszto I (19 3) Pathophysiology of human tears Int Ophthal Clin 13 119–147

Szapaszto I & Kerenyi G (1977) Skin replacement with Bioplast® fibrin in ophthalmology J Biomed Mater Res 11 799–809

Torok B Halmagyi Gy Lantos J & Kerenyi G (1975) Resorption of [1] I labelled fibrin Bioplast from the dorsal muscle of rats Biomat Med Dev Art Org 3 205–214

De Wecker L (1879) Chirurgie oculaire Octave Doin ed p ?02 Paris

Wolfe J R. (1873) On conjunctival transplantation from the rabbit Glasg med J 5 200–211

Wood C B Capperauld I & Blumgart L H (1976) Bioplast fibrin buttons for liver biopsy and partial hepatic resection Ann roy Coll Surg Engl 58 408–416

Author's address

Geza Kerenyi Ph D Novex Co Ltd

H 1056 Budapest Marcius 15 ter 1 Hungary

References

Miettinen R (1977) Incidence of uveitis in Northern Finland *Acta ophthal (Kbh) 55* [illegible]

Oksala A (1960) Ätiologie der Uveitis in Mittel Finnland *Docum Ophthal 14* [illegible]

Saari M Miettinen R & Alanko H (1975) Uveitis Report of a 10-year survey in [illegible] Finland *Canad J Ophthal 10* 356–360

Saari M & Nieminen H (1976) Fluorescein angiography and infrared [illegible] stereo technique for studying the ciliary body and iris Paper read in the 5th Co[illegible] the European Society of Ophthalmology 5–9 April 1976 Hamburg

Sixth Congress of the European Ophthalmological Society

The sixth meeting of the European Ophthalmological Society will be held in [illegible] England from 21st to 25th April 1980

The main theme for the scientific programme will be 'The Cornea in Health and [illegible]'

Subsidiary meetings on other subjects will be held in association with the main meeting [illegible] will include round table discussions and workshop sessions.

Papers should be submitted to Professor W S Foulds Department of Ophthalmology University of Glasgow Glasgow Scotland

Further information can be obtained from the Official Organiser Holland Organizing Centre 16 Lange Voorhout The Hague The Netherlands

The centenary meeting of the Ophthalmological Society of the United Kingdom will be held in London during the week preceding that of the European Society meeting in [illegible] to 18th April 1980

Further information for this meeting can be obtained from The Honorary Secretary Ophthalmological Society of the United Kingdom The Royal College of Surgeons Lincoln's Inn Fields London WC2 England

Postgraduate course of the University of California on Glaucoma

The postgraduate course of the University of California School of Medicine ([illegible] Glaucoma) is scheduled to be held at the St Francis Hotel on Union Square in San Francisco February 6–8 1980

For further information please contact Extended Programs in Medical Education [illegible] 569–U University of California San Francisco California 94143 or call (415) [illegible]

2nd International Congress on Vision and Road Safety Paris

is held from 20th to 22th November 1980 Night Driving is the conference theme [illegible] Lighting Vehicle Lights All night Road Signs and Signals and Reflectors are [illegible] themes. Numerous round table conferences

Information R Pansard General Secretary la prevention routiere internationale [illegible] 91310 Paris Montlhery France

24th Nordic Ophthalmological Congress 1979

ɪeld ɪn Oslo from 13th to 16th June 1979 with the cornea and ergophthalmo ɜs the main scientific topics The president was professor Jan ʏtteborg
e topic of the plenary meeting was organization and resource problems rning the combined visual and eye care services
ɪe plenary meeting closed with the *presentation of the K K K Lundsgaard Medal* gold medal and prize was to be awarded to the author of the best article in Acta ɔhalmologica volume 55 and 56 (1977 and 1978) The candidate was chosen on nmendation by all the Nordic professors as requested by the testamentator y articles according to my opinion were worthy of a gold medal The choice *Ole I Nissen* from the Eye Department Rigshospitalet Copenhagen



Nissen was selected because of his basic study of the disease process which is a ɪue to us all and which represents a scientific challenge namely glaucoma
ɪle I Nissen has developed a completely original method for the constant ısurement of the intraocular tension by means of a so-called suction-cup ɪination method in studies on rabbits and humans (*Acta ophthal (Kbh.)* 55 – 60)

Mogens Norn

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

From The Department of Ophthalmology (Head N Ehlers)
and The Blood Bank and Tissue Typing Laboratory (Head F Kissmeyer Nielsen)
Århus Kommunehospital University of Aarhus

CORNEAL TRANSPLANTATION AND HLA HISTOCOMPATIBILITY

A preliminary communication

By

NIELS EHLERS and FLEMMING KISSMEYER NIELSEN

A series of 222 cases of 7 mm penetrating corneal grafts were analysed with respect to the influence of HLA compatibility The degree of compatibility is random as no matching was done (HLA types unknown at time of operation *Consequently* most of the cases showed 3 or 4 incompatibilities.
The series was divided into seven diagnostic groups (keratoconus herpetic keratitis non herpetic keratitis stromal dystrophy endothelial dystrophy mechanical lesion and corrosion) In all groups there was a tendency towards better results among the compatible transplantation but only when considering the entire series could statistical significance be demonstrated The groups of 0–2 incompatibilities showed fewer rejection episodes or opaque grafts than the groups of 3 or 4 incompatibilities ($\chi^2 = 9.90$ $P < 0.005$) Comparing only the frequency of opaque grafts among the two groups the correlation was less significant ($\chi^2 = 3.66$ $P \sim 0.05$)

Key words cornea – graft – tissue typing

Resumé

The significance of an allograft reaction as the cause of graft failure has over years become more and more clear To day there is no doubt that immune reactions do occur (Grunnet et al 1976) and the importance of the major histocompatibility system the HLA system has been demonstrated (Ehlers Kissmeyer Nielsen 1973 1977 Gibbs et al 1974 Vannas et al 1976 Volker Dieben

k van Alphen 1978) It is probably fair to say that to date no series has been ted to prove the clinical value and show the place of HLA typing in future ace

ice 1968 we have performed 316 corneal transplantations of which 239 were nm penetrating grafts where in addition the HLA A and B types of donor recipient were determined Blood samples for typing were drawn after the plantation and no matching of donor and recipient was attempted The series therefore from a statistical point of view be considered suitable for an analysis e influence of HLA compatibility on the fate of the graft

the analysis of the series 17 cases had to be excluded for the following reasons surgical e 6 cases primary graft failure 4 panophthalmia 1 intraocular haemorrhage 1(un losed haemophilia) severe herpetic reinfection 2 glaucoma 2 and in 1 case antibodies in cipients serum directed against the donor (acute failure)

ie series of the remaining 222 cases is presented in Table I The diagnostic ip non herpetic keratitis includes all cases where none of the other diagnoses appropriate

onsidering the results the outcome of the transplantation was described as 1) r graft no rejection episodes 2) clear graft but definite rejection episodes aged by treatment (Ehlers & Bramsen 1978) or cured spontaneously (2 cases) 3) opaque graft. The cases were placed in the three categories by one of the iors (NE) without knowledge of the degree of compatibility between donor and pient

enerally the results were as could have been expected e g good in the groups eratoconus stromal dystrophy and mechanical lesion less favourable in the ups of herpetic keratitis non herpetic keratitis and endothelial dystrophy and ter unfavourable in the group of corrosions

Table I
Diagnosis of 222 corneal transplantations

Diagnosis	Women	Men	Total
Keratoconus	13	41	54
Herpetic keratitis	21	22	43
Non herpetic keratitis	34	18	52
Stromal dystrophy	4	11	15
Endothelial dystrophy	20	13	33
Mechanical lesion	2	8	10
Corrosion	1	14	15
Total	95	127	222

Table II
The overall result of 222 corneal transplantations.

	No of incompatibilities				
	0	1	2	3	4
Clear graft no rejection	2	5	36	59	[illegible]
Clear graft + rejection	–	–	1	9	[illegible]
Opaque graft	–	–	5	23	[illegible]

The overall result of the 222 transplantations (in june 1979) is shown in Table I. It is seen that most transplantations showed 3 or 4 incompatibility. Considering 0, 1 and 2 incompatibilities as rather favourable and 3 or 4 as unfavourable it can be shown by a 2×2 χ^2 table that rejection episodes or opaque grafts occur significantly more frequently among the incompatible transplantations than among the compatible ($\chi^2=9.20$ $P<0.005$). From a more clinical point of view one may distinguish between clear and opaque grafts and it can be found that more opaque grafts occur among the incompatible than among the compatible transplantations. However the significance is less ($\chi^2=3.66$ $P\sim0.05$).

In a following publication a more detailed presentation of our data will be given with calculated actuarial survival curves for the grafts. This has already been done for the first 140 of the cases (Ehlers & Kissmeyer Nielsen 1977). The same analysis confirmed the statements in the literature that the AB0 compatibility is of no significance.

The points to be discussed here are the possible consequences to be drawn from the accumulating evidence for the significance of histocompatibility for the result of corneal grafting. Evidently one way is to continue as we have done until now grafting without respect of the compatibility and regraft the inevitable failures. This attitude can by no means be disregarded when e g the favourable results obtained in keratoconus are considered. However rejections also occur in keratoconus and the problem increases with no of retransplantations and in other diagnostic groups. A more satisfactory approach would be to attempt to develop an organisational structure giving the possibility of selecting compatible donor-recipient pairs. This could be achieved by having a large pool of HLA typed recipients waiting for the right donor tissue or by having a large pool of HLA-typed donor corneas waiting for their recipient. For obvious reasons the latter possibility is preferable and we have therefore developed a freezing technique which will allow us to keep donor tissue at the temperature of liqued nitrogen for prolonged periods. These corneas have now been used successfully in several cases but the

ion still to be answered is how the advantage obtained by using compatible compares with the immediate postoperative problems introduced by using ned tissue

References

s N & Bramsen T (1978) Management of late corneal graft problems. *Acta phthal. k.) 56* 984–997

s N & Kissmeyer Nielsen F (1973) Influence of histocompatibility on the fate of the neal transplant. In *Corneal graft failure* Ciba Symposium 15 pp 307–319 Associated entific Publishers Amsterdam

s N & Kissmeyer Nielsen F (1977) Corneal transplantations and tissue compatibility *skr La 139* 2679–2682

s D C Batchelor J R Werb A Schlesinger W & Casey T (1974) The influence of ue typing compatibility on the fate of full thickness corneal grafts *Trans ophthal. Soc K 94* 101–106

unet N Kristensen T Kissmeyer Nielsen F & Ehlers N (1976) Occurrence of nphocytotoxic lymphocytes and antibodies after corneal transplantation *Acta ophthal bh) 54* 167–173

as S Karjalainen K Ruusuvaara P & Tuhkainen A (19 0) HLA-compatible donor mea for prevention of allograft reaction *Gra fes A ch kl n exp Ophthal 198* 217–222

er Dieben H J & Kok van Alphen C C (1978) Versuche der Gewebetypisierung von ender und Empfänger bei der Keratoplastik (Erste Versuche mit angepassten gewebe sierten Transplantaten bei Bad risk cases *Ber dt ch phthal Ges 75* 428–431

s add ess

Ehlers Eye Department

s Kommunehospital DK 8000 Århus C Denmark

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

Department of Ophthalmology (Head Jan Ytt borg)
University of Oslo Norway

ORGAN CULTURED DONOR MATERIAL FOR PENETRATING CORNEAL GRAFTS

A preliminary report

BY

ALBERT KOLSTAD

Fresh and undamaged cornea had unchanged endothelial cell density for four weeks in culture while eyes removed at autopsy suffered a cell loss. Acceptable cell density was found in eyes removed within eight hours, and this was confirmed by the results of 55 penetrating grafts using cultured autopsy material Storage in culture for more than one week seemed to influence the results unfavourably Immune reactions were not seen

Key words cornea – cornea/transplantation – tissue donors – organ culture

Traditional storage of the donor eye in a moist chamber at 4 C limits its use to [illegible] This may cause practical problems both for the patient and for the surgical te[illegible]

We have therefore looked for methods which will prolong the keeping q[illegible] of the donor corneae and have found that organ culture fulfills our requirem[illegible] and brings additional advantages

The culture of corneal tissue is no recent achievement. Its lack of vascular s[illegible] makes the cornea undemanding and successful culture of endothelium was [illegible] reported in 1928 (Matsui) Cultured donor material for penetrating grafts [illegible] suggested in 1948 but was a failure (Hoof 1948 Hoffman 1949)

During the last five years the interest for this approach has revived, and d[illegible] varieties of the culture method have been published (Summerlin et al 1[illegible] McCarey et al 1974)

have used the method which is common for other mammalian tissues culture n enriched with serum and kept at 37 C (Paul 1970)

Own Investigations

urvival of the endothelium in culture is a crucial question To answer this we used human eyes recently removed for intraocular malignancy Four buttons been punched from each cornea and these have been kept in culture for d periods of time before being stained with silver and the cell density mined (Oh 1963)

1 shows that cell density in non traumatized fresh eyes will remain practically anged for four weeks in culture

the endothelium has been subjected to mechanical trauma such as bending of ornea rows of silver stained cells will be seen corresponding to the folds in emets membrane Fig 2 After a few h in culture the undamaged neighbour

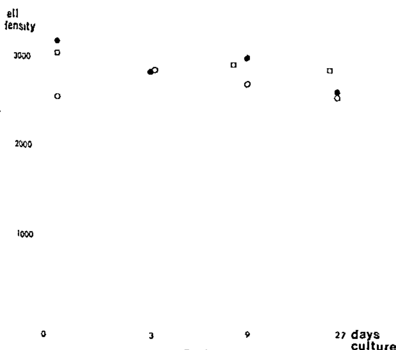


Fig 1

thelial cell survival in fresh undamaged corneal buttons kept in organ culture at 37 for o four weeks Cell density which is expressed in cells mm² is determined by counting s in the microscope after silver staining Filled circles open circles and squares denote corneal samples from three separate eyes remo ed for intraocular tumour

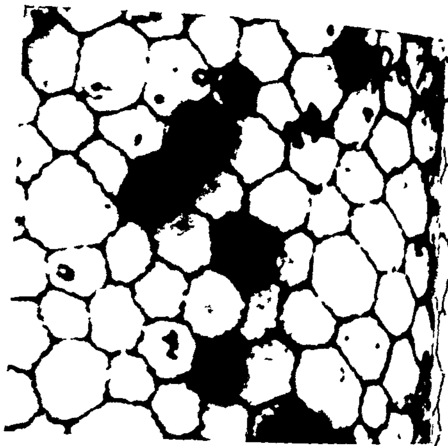


Fig 2
Silver stained endothelium of corneal button The dark cells are the result of [illegible] trauma. Cell density 2671 cells mm²

cells will expand to fill the space of the dead cells and debris thereby [illegible] regular hexagonal pattern Fig 3

Fig 4 shows the resulting fall in cell density during the first three days. The [illegible] now remaining are thought to be alive

The supply of fresh cornea from eyes removed for intraocular tumours [illegible] answer the demand for donor eyes We therefore use eyes removed in [illegible] with autopsy

We know that long delay between death and the removal of the eyes [illegible] compatible with a viable enothelium To determine how soon this destruction [illegible] place we have removed the eyes at various intervals after death and [illegible] surviving cells after three days in culture Fig 5 shows the decrease in cell density with increasing post mortem time

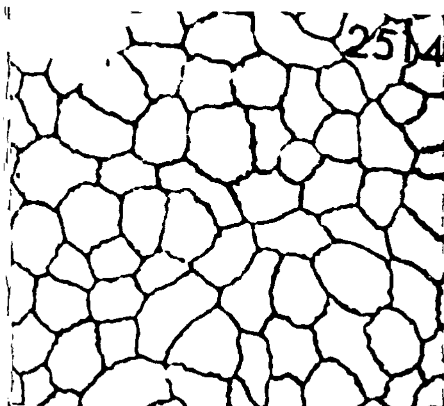


Fig 3

er stained endothelium of corneal button exposed to mechanical trauma after organ culture for 24 h The dark cells have disappeared Cell density 2514 cells/mm²

It is difficult to state the minimum cell density which can be accepted for donor iterial The mechanical trauma inflicted on the endothelium during surgery ould require cell density far above the limit for barrier function which probably n the neighbourhood of 2–300 cells/mm² (Fig 6)

If we choose 2000 cells/mm² as the lowest acceptable density for the donor dothelium Fig 5 shows that this was obtained by removing the eyes before 8 h st mortem

High donor age will adversely affect cell density (Laing et al 1976) When there s been a choice between eyes with low post mortem time and low donor age we ve preferred the former

The eyes are removed by a technician using unsterile clean instruments It is red in a sterile vessel in the refrigerator usually for no more than one h before ing prepared for culture

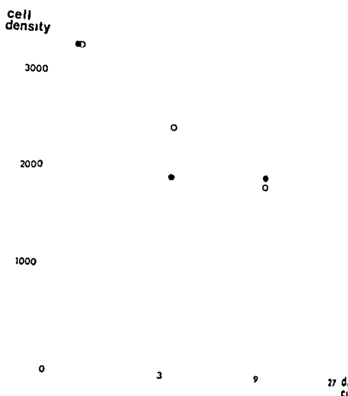


Fig 4
Endothelial cell survival in fresh corneal buttons exposed to mechanical trau

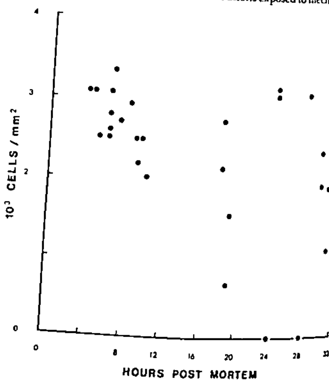


Fig 5
Endothelial cell survival in eyes removed at various time intervals after death. Cell density was determined by silver staining after three days in organ culture

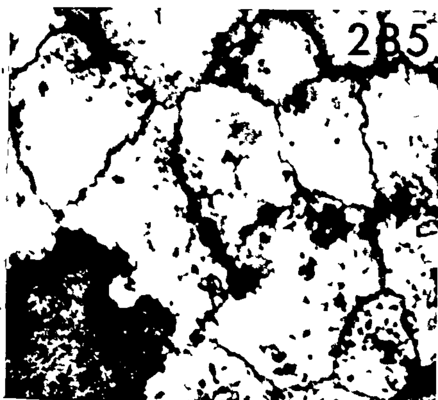


Fig 6

er stained endothelium of corneal button removed from patient with Fuchs dystrophy In te of maximal extension of endothelial cells area of Descemets membrane is left uncovered (lower left) Cell density 985 cells/mm²

First of all the epithelium is removed This will reduce the risk of infection and ll also prevent the epithelium from migrating onto the stromal section The nor button is then trephined to the correct diameter the section finished with a zor blade fragment and the button transferred to a test tube with 10 ml medium here it will settle endothelial side up on the bottom of the tube It is then placed in incubator at 37 C and the medium changed once a week

We use Eagle Minimum Essential Medium enriched with 10% inactivated human serum d with 2 mM l glutamine and 5 mg/100 ml Gentamycin added We prefer the Hepes buffer 0 mM) which will guarantee a correct pH in an open vessel We can therefore place the nor button in a Petri dish on the instrument table before surgery begins

The donor button is edematous when removed from the medium to be placed in e host Care should therefore be taken to place the sutures deeply close to

Table I
Results using organ cultured donor material for penetrating corneal grafts

Diagnosis	Number	Cloudy
Keratoconus	26	0
Bullous keratopathy	16	2(3)
Maculae corneae	5	1
Dystrophy	5	0
Rejection	2	0
Descemetocele	1	0
Total	55	3(4)

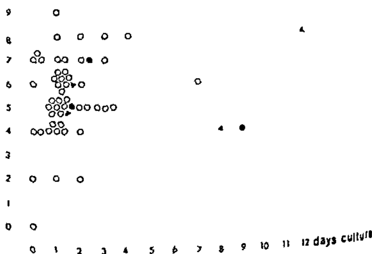


Fig 7
Results of penetrating grafts seen in relation to post mortem time and culture time of donor material. Open circles: clear. Filled circles: cloudy from start. Filled triangles: clear for more than six months, later gradual clouding

emets membrane The donor button will start clearing up during suturing and ually of normal transparency and thickness when pad and bandage is removed two days

e have not seen microbial growth during culture nor post operative infection ring the last three years we have used cultured donor material for 55 rating grafts Table I shows that three of these were cloudy from the first ge of bandage while another four turned gradually edematous after more six months

ost of the failures were in patients with bullous keratopathy where the host ea cannot be expected to contribute with any endothelial cells to the graft

g 7 demonstrates the relationship between post mortem time culture time and t failure Postponing removal of the donor eye up to eight hours after death did ect the results while storing the button in culture for more than a week ed to increase the number of failures We have for obvious reasons not ted to expand our material in this direction

or all practical purposes a limit to storage in culture of one week should be quate

e have had no case of immune reaction One possible explanation is that the or button discards the dead cells in culture and therefore presents a smaller genic stimulus to the host immune system

References

man R S & Messier P E (1949) Mechanism of corneal graft healing II Behavior of cipient and donor cells *Arch Ophthal 42* 148

f D (1948) Tissue research and cell culture of the cornea *Amer J Ophthal 31* 709

g R A Sandstrom M M Berrospi A R & Leibowitz H M (1976) Changes in the neal endothelium as a function of age *Exp Eye Res 22* 587

u J (1978) Uber die in vitro-Kultur des Endothels der Membrana Descemetii *Arch exp ilforsch 8* 533

arey B E & Kaufman H E (1974) Improved corneal storage *Invest Ophthal 13* 365

l O (1963) Changes with age in the corneal endothelium of normal rabbits *Acta ophthal kh) 41* 568

J (1970) *Cell and Tissue Culture* Livingstone London

merlin W T Miller G E Harris J E & Good R A (1973) The organ-cultured cornea n in vitro study *Invest Ophthal 12* 176

s add ess

n Kolstad Department of Ophthalmology
 l Sykehus Oslo 1 Norway

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

Department of Ophthalmology
(Heads S Faurschou E Goldschmidt and K [illegible])
Odense University Hospital Denmark

KERATITIS DENDRITICA

An epidemiological investigation

BY

K KAMP MORTENSEN and A. K. SJOLIE

One hundred and seven outbreaks of dendritic keratitis have been registered by the ophthalmologists over a 2 year period in a region with a population of approximately 446 000 persons The incidence was found to be 12/100 000/year
The average age of our sample was 46.5 years with the same age distribution for males and females and with a non significant predominance of males
No seasonal variation of dendritic keratitis was observed 50% of the patients had previously suffered from dendritic keratitis In 25% of the patients the onset of the disease had been preceded by an infectious disease while 6% were on local steroid therapy for non-dendritic eye diseases prior to the dendritic outbreak

Key words keratitis dendritica – herpes simplex virus (HSV) – incidence seasonal variation – recurrent infection

Herpetic keratitis is a frequent cause of infectious corneal disease in the [illegible] developed countries (Howard & Kaufman 1962) and produces with its tendency to recurrence a number of cases of serious visual sequelae Many experimental and epidemiological investigations have been carried out in order to obtain a better understanding of the frequent recurrences (Nesburn 1975 Baringer & S[illegible] 1973 Bastian et al 1972) HSV infection is a ubiquitary and frequently [illegible] infection thus serological investigations have demonstrated that [illegible]% of persons over the age of 20 years have circulating HSV antibodies (Nesburn 19[illegible] [illegible] approximately 90% of HSV infections are subclinical (Leopold & Sery [illegible]

he epidemiological investigations previously carried out have mainly been formed on hospital patients We have therefore found it of interest to carry out pidemiological study of dendritic keratitis in a well-defined geographical area.

Material and Methods

r a pilot study of 3 months duration registration was carried out of all patients ering from dendritic keratitis treated by the ophthalmologists in the county of en during the period 1/6 1976 to 31/5 1978 The county of Funen has a ulation of 446 223 persons (Danmark s Statistik 1977)

nformation was obtained of the age sex previous infectious diseases herpes alis or traumas and earlier attacks of ipsi or contralateral herpetic affection ally those patients who had been on local steroid treatment for an eye disease er than dendritic keratitis were noted

t test F test and χ^2 test were employed in the statistical analyses A significance level of was chosen

Results

otal of 107 outbreaks were registered giving an incidence of 12/100 000/year e patient suffered three attacks and five patients two attacks of dendritic keratitis ing the period of the investigation The registered patients (45 women 62 men) e found to have an average of 46 5 years range 4 to 86 years as can be seen in ble I Statistical analysis did not reveal any significant differences in the age tribution between the two sexes nor in the sex ratio However in the county of nen there are more women than men in the elderly age groups (Danmark s tistik 1977)

The distribution according to the seasonal outbreak of the disease is shown in ble II where there was no seasonal variation neither monthly nor quarterly us the disease arises evenly throughout the whole year

ifty three of the 107 outbreaks occurred in patients who had previously suffered m dendritic keratitis in the same eye while four occurred in patients who had viously had the affection in the contralateral eye as can be seen in Table I Only 5 patients had previously suffered from bilateral infection

Twenty seven patients stated that the corneal affection had been preceded by a mmon cold influenza or herpes labialis

Six patients were being treated with a local steroid for an eye disease other than ndritic keratitis prior to the present outbreak

Table I

Age and sex distribution as well as the recurrence rate of 10 outbreaks of dendritic keratitis

Age	Females	Females with earlier dendritic keratitis		Males	Males with earlier dendritic keratitis	
		Ipsilat.	Contralat.		Ipsilat.	Contralat.
0– 9	1	1	0	9	1	0
10–19	7	2	0	4	0	0
20–29	3	1	0	8	5	1
30–39	5	3	0	10	8	1
40–49	6	1	0	7	9	1
50–59	11	7	0	11	5	0
60–69	7	4	0	11	5	1
70–79	3	2	0	7	3	0
80–89	2	2	0	9	1	0
Total	45	23	0	69	30	4

Table II

The seasonal distribution of 107 outbreaks of dendritic keratitis registered over a period of 9 years

Month	Females	Males
January	4	9
February	3	5
March	3	8
April	4	1
May	4	5
June	8	6
July	1	8
August	6	5
September	6	3
October	4	4
November	1	5
December	1	3
Total	45	69

Discussion

ɔus epidemiological investigations regarding dendritic keratitis have mainly based on hospital materials thus Jonkers (1962) collected all the clinical and lant (sic) cases of herpes simplex corneae at the Rotterdam Eye Hospital g 19ɔ8 19ɔ9 and 1960 and found a frequency of herpes corneae of 1 out of patients Norn (1970) found among all the patients referred within a year period to the Department of Ophthalmology Kommunehospitalet nhagen the incidence of herpetic keratitis to be 5 9/100 000/year Leopold & (1963) as well as Caroleo (1970) have also carried out epidemiological studies ndritic keratitis based on hospital materials but the incidences are not stated ianc (197b) studied a total population of 800 000 persons and found that the nce of dendritic keratitis was 4/100 000/year while the *incidence in the* nt investigation is 12/100 000/year as based on the total population of a defined region Ophthalmological service is readily available within this area it must therefore be presumed that the majority of patients developing toms consulted an ophthalmologist and thus have been registered The ence found in the present investigation will be a minimum figure Due to t, transient symptoms a number of patients may not consult their physician pontaneous healing is seen in 10–25% (Thygeson 19ɔ6 Laibson 1964)

truschka (1968) found a predominance of patients in the age group 0 to 9 s Norn (1970) found a predominance in the age groups 0 to 9 and 40 to 60 s while Leopold & Sery (1963) observed the highest incidence between the ages 3 and 60 years A predominance of men has been seen in all the studies The ent investigation shows no significant difference in the sex distribution between two sexes even though there is a non significant predominance of men The distribution of the population within the county of Funen is not similar for both and women as there are more women than men in the elderly age groups In present material there are too few patients in the age group 0 to 9 years in ion to the age distribution of the population within the area In addition there relatively too many men over the age of 40 years A possible explanation of the stration of fewer children in this material as compared to those of earlier studies be that children in contrast to adults are more often referred to a hospital to the difficulty in treating them For this reason children will comprise a tively larger percentage of a hospital material than of the registered patients in a erial based on the total population The large number of middle aged and rly patients found in the other investigations may be caused by a poorer nune response in the elderly age groups

seasonal variation has been observed in the outbreak of herpetic keratitis in ny of the previous investigations In particular attention has been given to the

possibility of an increased incidence in the winter months, together with frequent upper respiratory tract infections. The present investigation has demonstrated in good general agreement with Norn (1970) and Caroleo [illegible] there is no seasonal variation. The rate of recurrent attacks is high [illegible] Jonkers (1962) and 61% in that of Norn (1970). This investigation has shown that 50% of the patients had ipsilateral recurrence while 4% had contralateral recurrence. The high rate of ipsilateral recurrence supports the theory that recurrence results from an activation of virus elements, which are dormant in the trigeminal ganglion.

The present investigation does not permit any definite conclusions [illegible] as to the trigger mechanism responsible for the activation of these virus elements.

Acknowledgments

The authors would like to thank the ophthalmologists Drs. D. Coucle, H. O. B[illegible], T. Haghfeldt, A. M. Land, B. Moestrup, G. Pouplier, A. Quist, J. A. Rak, T. [illegible], H. Ravn Sorensen, J. Wegener and N. Willumsen for participating in the investigation.

References

Baringer J. R. & Swoveland P. (1973) Recovery of herpes-simplex virus from human trigeminal ganglions. N. Eng. J. Med. 288, 648–650.

Bastian F. O., Rabson A. S., Yee C. L. & Tralka T. S. (19[illegible]) Herpes virus hominis [illegible] from human trigeminal ganglion. Science 178, 306–307.

Caroleo D. (1970) Epidemiologia della cheratite erpetica. Ann. Ottal. 96, [illegible]

Danmarks Statistik (1977) 81, 19.

Howard G. M. & Kaufman H. E. (1962) Herpes simplex keratitis. [illegible]

Jonkers G. H. (1962) Statistical investigation on the occurrence of herpes simplex [illegible] Ophthalmologica 144, 405–408.

Laibson P. R. & Leopold I. H. (1964) An evaluation of double-blind IDU therapy in [illegible] of herpetic keratitis. Trans. amer. Acad. Ophthal. Otolaryng. 68, 22–3[illegible]

Leopold I. H. & Sery T. W. (1963) Epidemiology of herpes simplex keratitis. [illegible] 2, 498–503.

Nesburn A. B. (1975) Recurrence in ocular herpes simplex infection. Int. Ophthal. Clin. [illegible] 101–110.

Norn M. S. (1970) Dendritic (herpetic) keratitis. Acta Ophthal. (Kbh.) 48, 91 [illegible]

Pietruschka C. (1968) Zur Epidemiologie und Therapie des Herpes Corneae. [illegible] Cesk. Oftal. 23, 1249–1254.

Ribaric V. (1976) The incidence of herpetic keratitis among population. [illegible] 19–22.

Thygeson P., Kimura S. J. & Hogan M. J. (1956) Observations on herpetic keratitis and keratoconjunctivitis. Arch. Ophthal. (Chic.) 56, 3[illegible]

Authors' address:

K. Kamp Mortensen and A. K. Sjolie

Department of Ophthalmology, Kolding Sygehus, DK-6000 Kolding, Denmark

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

*Department fOphthalmology (Head E Greger en) and
Section of Ca diology (Head A Tybje g Hansen)
Medical Department B Rigsho p talet C penha en*

PRESENILE CORNEAL ARCUS IN HEALTHY PERSONS A POSSIBLE CARDIOVASCULAR RISK INDICATOR IN YOUNGER ADULTS

BY

L. VARNEK P SCHNOHR and G JENSEN

The results of blood lipid screening of 900 39–49 year-old participants with incidentally diagnosed corneal arcus from the Copenhagen City Heart Study are reported

A large sample of study participants of the same age-group without arcus served as controls

In general the arcus group had higher se -cholesterol than the control-group ($P<0.01$ in males and <0.02 in females) The se triglycerides in the two groups did not differ

A trend towards *positive correlation between* arcus-intensity and cholesterol level was demonstrated by non parametrical statistics By use of the same statistics an association between lipids and arcus-localisation was searched out

Dense *arcus in* the nasal *lower* limbal *regions* occurred *together with* hyper cholesterolemia more often than did a marked upper outer arcus

Consequently based on the present investigation se -cholesterol screening of middle aged persons with marked lower nasal arcus seems justified, since hypercholesterolemia might will be an underlying cause

Finally the importance of diagnosing and treating hypercholesterolemia is briefly discussed

Key words corneal arcus slit lamp grading – pre alence in middle age – hyperch lesterolemia – se triglycerides – se glucose – arcus-intensity – arcus localisation.

Our present knowledge regarding corneal arcus in relation to lipid abn... and cardiovascular disease/death dates from several sources. A connection between arcus in pre middle age and acute myocardial infarction has been evidenced ... large scale prospective population study the Western Collaborative Study ... (Jaeger & Eisenhauer 1977) whereas others have reported a lack of ... between atherosclerosis verified by authopsy and arcus prevalence ... 1967)

Screenings for arcus prevalence and arcus-degree have established strong ... lations between hyperlipidemia especially hypercholesterolemia, and ... (Jaeger & Eisenhauer 1977) The same investigators further stressed the ... importance of lower (= inferiorly located) arcus in relation to blood lipid abn... lities

Reports on lipid screening on normals with incidentally diagnosed corneal ... are rare and the results are controversial (Parwaresch et al 1976)

To our knowledge no earlier attempts have been done on a larger scale to ... the clinical importance of both arcus intensity and prevailing arcus-localisation ... symptom free individuals

So the aim of the present study is to analyse the correlations between ... lipids in this case cholesterol and triglyceride and corneal arcus in a g... presumed healthy pre middle aged persons

Material

From the total group of participants in the Copenhagen City Heart S... (abbreviated CCHS) aged 39—49 years in 1977 those persons were selected ... study in whom a corneal arcus was macroscopically diagnosed by the partici... cardiologists Before inclusion into the study the persons underwent ... examination to verify the arcus and to exclude persons with gross local ... pathology from the study

As blood parameters the non fasting values of se-cholesterol se-trigly... se glucose (mmol/l) were chosen

Out of 270 persons 200 persons 115 males and 85 females, participated ... study and constitute our arcus group

As blood sample-controls served the total group of participants in the ... aged 39—49 years in 1977 (1440 males and 1705 females) They were ... the data registration available accepted as controls because of the low ... prevalence in the group about 6 8%

Methods

liminary procedures

ophthalmic investigations were performed by one of us (L. Varnek) over seven hs Prior to the slit lamp examinations every person received a letter ining why where and when to meet. Eighty five % of those who received the attended for examination

amination proper

imp examination was performed on right eyes only by the use of a Haag Streit mp (type 900) supplied with tungsten light magnification × 6 and fixed diffuse 6 V illumination The corneal arcus was given a total score ranging 0–12 and a quadrant score ranging from 0–3 in any of the four quadrants r lower nasal temporal based on both surface-extension and surface isity of the arcus Fig 1 gives examples of this scoring system

ially the cornea under investigation was photographed with a Zeiss slit lamp ra (Ectachrome magnification × 2)

oring methodology remarks

iodological difficulties in obtaining an "exact" arcus scoring were experienced both arcus-surface-extension and arcus surface intensity varied along the al circumference

ie subjective way of scoring which has been employed by the single observer in studi was controlled by means of the photographs taken

egistration

person in the study received four registration sheets one for name address etc one for tr tion of known diabetes earlier cerebral strokes or myocardial infarction one for tratrn of the blood sample analyses (these three sheets were taken as photocopies from

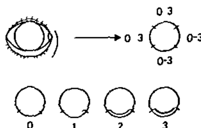


Fig 1

ematic Illustration of the employed arcus-scoring system Total score values obtainable 12 No arcus = score 0 Weak arcus = score 1 Intermed = score 2 Strong arcus = score 3

the journals on the persons in the CCHS) and finally one for corneal arcus c[illegible] supplementary corneal remarks when necessary

Because of the separated sheets the lipid values of the persons [illegible] arcus score were unknown to the slit lamp investigator

Results

To assess significance of the observed associations a probability level of $P <$ [illegible] used

In the arcus group the mean age of the examined males was 46 5 years [illegible] years) and females 46 6 years (SD ± 3 1 years)

The two sexes had the same mean total arcus score value of 5 3

Comparing the whole arcus group with the controls a significantly higher [illegible] cholesterol level was demonstrated in the arcus group and in both sexes (see Fig [illegible] a) ($P < 0.01$ in males and < 0.02 in females)

In the two sexes there were no differences between arcus group and controls [illegible] concerns triglyceride and glucose levels (see Fig 2 b and c)

Correlations between arcus intensity (score 0–19) and se[illegible] analyses were calcu[illegible] lated by non parameterical statistical methods (Kruskal Wallis) (see Table [illegible]) Especially in females but also in males there was a trend towards a p[illegible] correlation between arcus-degree and cholesterol values which in the females nearly reached significance ($P < 0.074$)

Table II shows a significantly higher frequency of high cholesterol values [illegible] female group of high arcus scorers as compared with low arcus-scorers ($P <$ 0 01) but not in the corresponding male groups ($P > 0.05$)

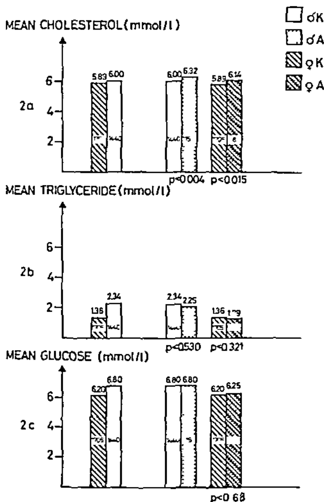
Finally we wanted to establish whether arci with shifting localisations (up[illegible] temporal nasal) were equally often combined with and thereby the [illegible] expression of an underlying hypercholesterolemia.

The arcus areas selected for investigation were the upper lower nasal and temporal limbal quadrants

Again by use of the non parametrical Kruskal Wallis analysis (see Figs 3[illegible]) and based on the arcus scoring system from 0–3 in the quadrants earlier de[illegible] correlations between increasing score values at the four chosen limbal s[illegible] blood sample analyses in either sex were calculate and in total 24 P values [illegible]

Out of the 24 possible correlations examined three significant correla[illegible] cholesterol attached were established

1 Females showed a positive correlation between degree of nasal arcus [illegible] cholesterol values ($P < 0.05$)



Figs 2 a–c

…mn diagrams showing the mean arithmetic values for non fasting cholesterol (Fig 2 a) …lyceride (Fig 2 b) and glucose (Fig 2 c) in mmol/l in males and females of the arcus and …trol groups. ☐ males control group ☐ males arcus group ☐ females control …up ☐ females arcus group The two first columns (left) show the absence of a …difference in se-cholesterol and the presence of a sex-difference as for se-triglyceride …d se-glucose In the last four columns (right) sample means of arcus and control groups …apposed (see text) Numbers within columns shows the size of the group under …sideration. Numbers at top of the columns show the arithmetic mean value of the relevant …od sample Number at bottom of columns obtained *P* values in comparing sample means (Student's *t* test, computer assisted)

Table I

Correlation between arcus intensity and cholesterol triglyceride and glucose in [illegible] females in the arcus group A slight positive trend between arcus-score and [illegible] cholesterol is demonstrated especially in females statistically insignificant, however [illegible] level (Kruskal Wallis)

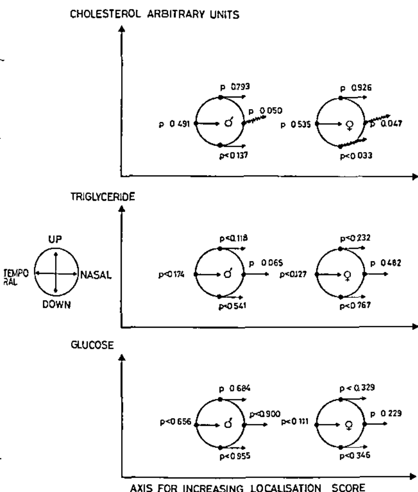
Cholesterol		Triglyceride		Glucose	
Males	Females	Males	Females	Males	Females
0 128	*0 074*	0 780	0 160	0 431	0 [illegible]

Correlation between se analysis and arcus intensity *P* values

Table II

Absolute and relative occurrence of se -cholesterol values higher than 7 0 mmol/l [illegible] higher than 8 0 mmol/l (right) in males and females of the arcus group further [illegible] high and low total arcus scores The relative frequency of high cholesterol values [illegible] high arcus score group" is impressive Significance levels (χ^2 tests) between low and high arcus scorers are shown in brackets

	No of persons	Persons with se -cholesterol > 7 0 mmol/l		Persons with se -cholesterol > 8 0 mmol/l	
		No	%	No	%
Males					
Low arcus score 0–5	58	9	15 8	2	3 5
			(0 1 > *P* > 0 05)		(*P* > 0 1)
High arcus score 6–12	57	20	35 1	6	10 5
Females					
Low arcus score 0–5	55	3	5 5	1	1 8
			(0 01 > *P* > 0 001)		(0 05 > *P* > [illegible]
High arcus score 6–12	30	10	33 3	4	13 3



Figs 3 a–c

-parametrically calculated correlations (Kruskal Wallis analysis computer assisted) in es and females between relevant serum values and increasing arcus score in the four bal quadrants The 6 circles symbolize limbal rings of right eyes The P value for any given relation (sex/se analysis/arcuslocalisation) is placed in relation to an arrow originating m the localisation under study which for illustsrative purposes depicts correlation ($P \leq$ 5) if it is ascending and zigzagged and lack of correlation ($P > 0.05$) if it runs horizontal and is straight

Table III

Occurrence of absolute and relative se.-cholesterol values higher than 7.0 mmol/l in males of the arcus group, divided into "high" and "low" nasal arcus scorers. Significance level (χ^2-test) between "low and high" nasal arcus scorers is shown in the bracket.

Males	No. of persons	Persons with se.-cholesterol > 7.0 mmol/l	
		No.	%
Low nasal arcus score 0–1	87	16	18.4
			(0.02 < P < 0.05)
High nasal arcus score 2–3	27	12	44.4

Table IV

Occurrence of absolute and relative se.-cholesterol values higher than 7.0 mmol/l in females of the arcus group divided into "high" and "low" nasal arcus scorers. Significance level (χ^2-test) between "low and high" nasal arcus scorers is shown in the bracket.

Females	No. of persons	Persons with se.-cholesterol > 7.0 mmol/l	
		No.	%
Low nasal arcus score 0–1	62	5	8.1
			(0.02 < P < 0.05)
High nasal arcus score 2–3	22	8	36.4

Table V

Occurrence of absolute and relative se-cholesterol values higher than 7 0 mmol/l in females of the arcus group divided into high and low inferior (lower) arcus scorers Significance level (χ test) between low and high inferior arcus-scorers" is shown in the bracket

Females	No of persons	Persons with se-cholesterol > 7 0 mmol l	
		No	%
Low inferior arcus score 0–1	37	2	5 4
			($0\ 02 < P < 0\ 05$)
High inferior arcus score 2–3	47	11	23 4

Males showed a positive correlation between degree of *nasal* arcus and cholesterol ues ($P = 0\ 05$)

Females also showed a positive correlation between degree of *inferiorly situated* us and cholesterol values ($P < 0\ 04$)

bles III IV and V give support to the three significant correlations

The other 21 P values none of which were significant at a 0 05 level are shown in s 3 a–c The uniformly high P values (cholesterol attached) in relation to *upper* us formation are remarkable

Discussion

ost authors agree upon the existence of a correlation between arcus and percholesterolemia in younger age groups this association however disappears the older age groups where *local* corneal factors are thought to dominate in cus formation (Cogan 1974 Immich et al 1967 Parwaresch et al 1976 osenmann et al 1974)

garding the intermediate age groups opinions are diverging one group of thors claiming lack of significance (Immich et al 1967) another an obligate -existence of underlying lipid abnormalities (Parwaresch et al 1976) The latter ew however cannot be true since the prevalence of lipid abnormalities in the

total population (Wood et al 1972) does not at all reach that of arcus in the age group under study (39–49 years) (10–25% Forsius 1954 Jaeger & Eisenhauer 1977)

As concerns our own results the higher mean cholesterol levels in the male and female arcus groups are in agreement with results obtained by others (Cogan [illegible] Immich et al 1967 Jaeger & Eisenhauer 1977 Rosenmann et al 1974). While the total occurrence of hypercholesterolemia (se -cholesterol higher than 7 0 mmol [illegible]) in the arcus group is 20% (males 25% females 15%) (Table II) the [illegible] occurrence of hypercholesterolemia (Fredericksson II) in the general population is only 3 7% (Wood et al 1972)

Our correlation between arcus *intensity* and se -cholesterol, demonstrated [illegible] women only has gained less support in the literature. Forsius (1954) [illegible] increasing arcus intensity in both sexes to increasing blood cholesterol levels [illegible] only in his younger arcus groups In contrast Parwaresch et al (1976) [illegible] mentioned a lack of correlation between arcus-degree and blood lipids

Regarding the significance of arcus-*localisation* we found in accordance [illegible] suggestions in the literature (Forsius 1954 Jaeger & Eisenhauer 1977) a [illegible] correlation between dense lower arcus formation in females but not in males [illegible] high cholesterol values

We also think that the obvious lack of correlation between dense *upper* arcus [illegible] blood cholesterol levels deserves to be mentioned

Our most significant finding the correlation between marked arcus formation nasally in both sexes and high cholesterol values seems to be entirely new To our knowledge at least a distinction in importance between *nasally* situated arcus and [illegible] situated *elsewhere* has not previously been put forward

Since several limbal affections preferentially develop nasally e g pterygia pingueculae and spheroid degeneration possibly because of local degenerative factors in this region degenerative corneal changes might be responsible for the above associations However a lack of underlying degenerative corneal changes [illegible] arcus formation speaks against this (Cogan & Kuwabara 1959)

Summing up *marked* arcus formation in both sexes but especially in females and *marked nasal* arcus in both sexes should arouse suspicion of underlying hyper-cholesterolemia at least within the age group studied (39–49 years)

Since familiar hypercholesterolemia is associated with serious cardiovascular complications if untreated (Research Committee 1971 Coronary Project [illegible]) [illegible] should deserve early recognition

The cholesterol lowering effect of diet restrictions and lowering of caloric [illegible] is well established (Parwaresch et al 1976) but during the last few years [illegible] 1978) treatment with lipid lowering agents especially atromidin and [illegible] considered safe and free from disturbing side effects has come into [illegible]

ased of stated connexion with gall-stones (atromidin) and skin disorders n) and suspected connexions with gastro-intestinal malignancies precipitation rdiac arrhythmias and sudden death

though the clinical benefice of diagnosing hypercholesterolemia in an other symptom free person thus seems debatable better and safer treatment might me available for future use and until then the above mentioned lipid lowering ts are still in widespread use

would therefore as a prophylactic precaution against hypercholesterolemia est lipid screening on any pre middle aged person with marked especially ly marked corneal arcus

References

n D G (1974) The corneal arcus N Engl J Med 291 1356

n D G & Kuwabara T (1959) Arcus senilis its pathology and histochemistry Arch thal (Chicago) 61 553–560

mittee of Principal Investigators (1978) Clofibrate a final verdict? Lancet I 1131–1132

nary Drug Project Research Group (1975) Clofibrate and niacin in coronary heart ease J Amer med Ass 231 360–380

ius H (1954) Arcus senilis corneae Acta ophthal (Kbh) Suppl 42 1–78

ips of Physicians of the Newcastle upon Tyne Region (1971) Trial of clofibrate in the atment of ischaemic heart disease Brit Med J 4 767–7 5

ch H Jansen H H & Pisani K (1967) Die beziehungen zwischen Arcus Lipoides der rnhaut und Arteriosklerose Vergleichende Untersuchungen von 500 Sektionsfällen m Wschr 45 1017–1020

er W & Eisenhauer G G (1977) Der diagnostische Wert der Arcus corneae als Hinweis f Lipoidstoffwechselstorungen Klin Mbl Augenheilk 171 321–330

aresch M R Haacke H M Mader Ch & Godt Ch (1976) Arcus lipoides corneae und perlipoproteinamie Klin Wschr 54 495–497

D (1966) Modellversuche zur Entstehung des Arcus Lipoides corneae im Geldiffusionsrsuche nach Ouchterlony Albrecht v Graef s Arch klin exp Ophthal 170 123–126

earch Committee of the Scottish Society of Physicians (1971) Ischaemic heart disease A condary prevention trial using clofibrate Brit M d J 4 775–784

enman R H Brand R J Sholtz R I & Jenkins C D (1974) Relation of corneal arcus to rdiovascular risk factors and the incidence of coronary disease N Engl J Med 291 399–1394

od P D S Stern M P Solvers A Rea en G M & Groeben J v d (1972) Prevalence of lasma lipoprotein abnormalities in a free living popoulation of the Central Valley alifornia Circulation XLV 114–126

thor s add ess

Larnek, Eye Department

ntofte Hospital DK 2900 Hellerup Denmark

Table I

Thickness of the precorneal fatty layer in normal eyes assessed by interference study Sex and age incidence

Age	Average ± SEM (nm)	≥200 nm (%)	No. of eyes
10–20	133 ± 20	18	17
20–30	95 ± 10	0	19
30–40	123 ± 16	10	10
40–50	97 ± 13	0	10
50–60	106 ± 11	9	[illegible]
60–70	100 ± 8	[illegible]	43
70–80	93 ± 6	5	39
80–90	104 ± 10	4	6
Total	102 ± 3	5 3	[illegible]06
Females	100 ± 4	5 5	1[illegible]8
Males	105 ± 6	5 1	[illegible]8

precorneal film and in the presence of a suitably thick fatty layer the interference phenomenon itself can be seen curved and winding brilliantly coloured lines across and beyond the illuminated area

Red interference colours indicate a not less than 200 nm thick fatty layer [illegible] waves extinguished being displaced half the wave breadth Blue = 4000 Å [illegible] 2000 Å = 0 2 μm = 200 nm)

If red interference colour is not directly visible the examiner must place a [illegible] on the outside of the patient s lower lid and move this upwards at a slow rate [illegible] the red interference phenomenon occurs If the normal palpebral fissure is reckoned to be 12 mm high and the red colour does not appear until the fissure has been narrowed to 6 mm for instance the fatty layer is estimated to be half of the stated 200 nm i e 100 nm thick The area of the mirror reflected on the cornea is [illegible] mm high and reaches the centre of the cornea by the fixation employed.

The principle of the semiquantitative method is in other words that of narrowing the palpebral fissure until *red* interference colour appears. The [illegible]ness of the fatty layer (Table I) is calculated on this basis. In patients with [illegible] measurement takes place after distension of the palpebral fissure to a [illegible] 12 mm

In rare cases with a very thick fatty layer this is seen as a cloudy [illegible] layer *with no* interference colours (Mc Donald s crumpling phenomenon [illegible]

he fatty layer is thin weak blue interference lines may be seen prior to ence of the characteristic red lines on further narrowing of the palpebral

Donald (1969) used a Goos lamp placed behind the slit lamp for illumination of the n, but undertook no quantification The Goos lamp has the advantage of allowing the cornea to be reflected successively However in the absence of such an arrangement ove mentioned ground glass plate is a very useful aid

eriments on oneself are carried out using the concave mirror of a microscope with a + 6 cal lens attached – mounted on an X ray projector screen according to Mc Donald or from of the bulb of a lit Goos lamp

tistical method employed is that of Students *t*-test. The results are expresed as s ± SEM (standard error of mean) $P < 0.05$ is considered significant

Material

series of patients studied come from an eye department, an ophthalmic patient department and the authors own ophthalmic practice The normal s comprises untreated patients with no conjunctival complaints referred for ine examination A total of 206 normal eyes from 166 subjects were examined age incidence is shown in Table I The total series comprises 303 subjects with ether 354 pathological eyes The diagnoses are shown in Table II

Results

mal series

le I shows that in normal eyes the fatty layer has an average thickness of 102 ± n (about 0 1 µm) In no more than 5 per cent was the fatty layer so thick that red rference was visible at wide-open palpebral fissure (≧200 nm) The normal es was evenly distributed corresponding approximately to a gaussian bell ped curve (Fig 2) No sex difference was noticed The amount of fat was ependent of age an observed tendency towards an increased amount in n agers was not significant (Table I)

Twenty duplicate determinations gave a coefficient of variation of 12 7 per cent.

thological series

might be expected an increased amount of fat was observed in patients ffering from chronic squamous blepharitis with greasy marginal scales The ckness of the fatty layer averaged 129 nm Maximum thickness was seen in 22 per nt (Table II)

Table II

Thickness of the precorneal fatty layer in 354 pathological eyes compared with [illegible] (cf Table I) The last column but one indicates in per cent the number [illegible] maximum fatty layer with red interference phenomenon with [illegible]

Diagnosis	Average ± SEM (nm)	*P*	≥ 200 nm (%)	[illegible]
Chron squamous blepharoconj	129 ± 8	< 0 001	[illegible]	[illegible]
Contact lens hard	187 ± 8	< 0 001	[illegible]	[illegible]
Contact lens soft	147 ± 18	< 0 0[illegible]	40	[illegible]
Cat postop 4–6 days old	197 ± 2	< 0 001	94	[illegible]
Acute infectious conj	193 ± 3	< 0 001	85	[illegible]
Chron infectious conj	164 ± 7	< 0 001	41	[illegible]
Obs for infect conj	169 ± 14	< 0 01	5[illegible]	[illegible]
Chronic simple conj	124 ± 13	n s.	[illegible]	[illegible]
Keratoconj sicca	163 ± 9	< 0 001	40	[illegible]
Recurrent corneal erosion	48 ± 13	< 0 001	0	[illegible]
Corneal transplantat > 4 months	108 ± 11	n s	0	[illegible]
Corneal disorders other	137 ± 19	n s	44	[illegible]
Iritis	200 ± 0		100	[illegible]
Episcleritis	106 ± 20	n s.	[illegible]	[illegible]
Allergy (conj palp)	95 ± 14	n s	0	
Sundry	161 ± 7	< 0 001	33	[illegible]

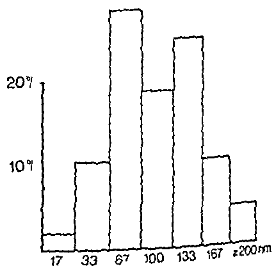


Fig 2

Thickness of precorneal fatty layer estimated by interference studies in [illegible] normal [illegible] Mean 102 nm Abscissa Thickness of fatty layer Ordinate Number of normals [illegible] layer thickness concerned expressed in per cent.

ring of contact lenses whether hard or soft, was in most cases associated with sed amounts of fat both across the lens itself (pre-contant lens film) and e the lens and also after removal of the lens The contact lenses looked clean itively few cases only In these the fatty layer was as in normal eyes (in 9 per f hard lens wearers and in 27 per cent of soft lens wearers)

amount of fat was even more frequently increased in patients with acute ial infectious conjunctivitis and panophthalmia The method can be em d to distinguish between bacterial conjunctivitis and a sterile conjunctiva (obs fectious conjunctivitis in Table II)

hick fatty layer was seen a few days after cataract extraction This may be due r to the postoperative inflammation or to the oil treatment given (ultralanum chloramphenicol 5 mg fluocortoloni pavalas and 2 mg chloramphenicol in ous oil to 1 g)

keratoconjunctivitis sicca the amount of fat was increased in the cases with iated clinical signs of bacterial infection The same was true for patients with r ophthalmicus meibomitis marginal keratitis exophthalmos facial nerve and ectropion if concurrent signs of bacterial infection were present

tients with chronic conjunctival complaints (simple conjunctivitis) on the other l had a normal fatty layer The same was true for patients with allergic unctivitis epiphora ptosis episcleritis corneal disorders with no signs of rial infection (dendritic keratitis corneal opacity keratoconus) several weeks

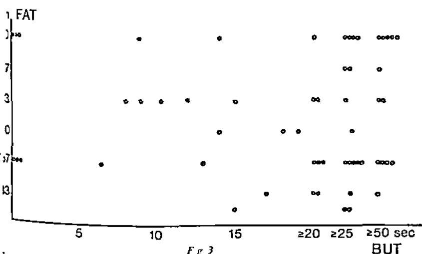


Fig 3

lation between stability of the precorneal film and thickness of the fatty layer (73 eyes) Dot agram Abscissa Break up time in seconds Ordinate Thickness of fatty layer of precorneal film in nm

Table III

Thickness of the precorneal fatty layer Experimental studies Influence ... (liquid paraffin 20% – petroleum jelly 80% mixture) before and after ... small number of meibomian glands ultracortenol drops (acetate 0.5% ... suspension) silicone oil dropos (dimetikon 7 and oftasiloxan ... polyethylene glycol Lab Kaysetberg France) 8) Immediately after ... 20 min later A total of 84 experiments

	Before average ± SEM	After average ± SEM	P	
Ointment	76 ± 8	200 ± 0	<0.001	
Squeezing of meibomian glands	57 ± 7	200 ± 0	<0.001	
Cocaine 2%	90 ± 10	113 ± 17	n.s.	
Ultracortenol drops	123 ± 23	120 ± 21	n.s.	
Silicone oil	142 ± 12	1 ± 1	<0.001	
Awakening	200 ± 0	150 ± 10	<0.001	

postoperatively (cataract corneal transplantation) The small number of ... showed increased amounts of fat

A reduced thickness of the fatty layer was noticed in one group only ... with a diagnosis of recurrent corneal erosion

The stability of the precorneal film might be conceived to depend on thickness of the fatty layer Fig 3 shows however that examination of ... 39 patients revealed no correlation between break up time and thickness of the layer

Experimental studies

Application of ointment gave a maximum fatty layer on the precorneal film as the ointment had melted and been distributed over the cornea. Thicker but ointment in lumps effected no interference But after some blinks the ointment was distributed in an even layer giving maximum interference (Table III) Maximum thickness of the fatty layer could be seen upto 24 h after the application For squeezing of a small number of meibomian glands the secretion ... formed a maximally thick precorneal fatty layer

The following factors seemed *not* to alter the fatty layer of the precorneal ... 2% cocaine eye drops ultracortenol acetate suspension (0.5% prednisolone ... methylcellulose (mucomimetic) intense rubbing of the eyelids with the ...

The fatty layer could be *reduced* by instillation of silicone oil a water ... compound reducing the aqueous phase of the precorneal film ...

, ki 1977) The interference phenomenon disappeared completely immedi fter instillation. Five to ten min later fairly small islands occurred with ed lines which gradually spread with the tear fluid across the cornea

xperiments on myself the amount of fat was found to be at a maximum the nt the eyes were opened in the morning after sleep (Table III) In seven out observations the precorneal film was covered by a silky granular layer ig no interference phenomenon as a thick fatty layer The results of the t investigation bore out this view maximum interference phenomenon in these cases occurred after few minutes or directly by ectropionising the n the remaining 15 cases maximum interference phenomenon was observed liately The fatty layer returned to normal between 20 and 60 min later and ned normal the rest of the day

l staining with alcian blue showed that mucus gives no interference pheno- n and that with fluorescein dry spots on the cornea likewise cause no rence phenomenon

on the conjunctiva on contact lenses and on eye prostheses can yield distinct rence phenomena

Discussion

onald (1969) characterized the fatty layer on the cornea as a layer proceeding the lid margins with a drape-effect In my judgment the fatty layer and the erence phenomenon diffusely cover the precorneal film By narrowing of the bral fissure the phenomenon is recognizable all over not only along the lid ins but also across the centre This observation led to development of the od first described in this paper for semiquantitative measurement of the ness of the fatty layer

ording to clinical experience the method is useful for assessing fat induced les in wearers of *contact lenses in applicants for contact lenses* and in patients blepharoconjunctivitis

cterial conjunctivitis is always associated with presence of an increased amount t This is not directly due to the neutrophilic granulocytes which are not ainable In the mucous thread in the inferior fornix the fat gathers to form s in vacuoles while the inflammatory cells are found scattered in the mucous d (Norn 1974)

increased amount of fat may thus either be due to a primary increased fat luction (blepharitis contact lens) or be secondary to bacterial conjunctivitis latter can be demonstrated with greater certainty by a quantitative cytological

conjunctival tear fluid examination or by vital staining with tetra[illegible]
blue (Norn 1974)

In the present investigation observations were made sugg[illegible]
amount of fat on the precorneal film may cause recurrent corneal e[illegible]

References

Mc Donald J E (1969) Surface phenomena of the tear film *Amer J Oph[illegible]*

Mishima S & Maurice D M (1961) The oily layer of the tear film and its [illegible] corneal surface *Exp Eye Res 1* 39–45

Norn M S (1963) Fatty substances on conjunctiva *Acta ophthal (Kbh) 41* [illegible]

Norn M S (1974) *External Eye Methods of examination* Scriptor Copenhagen, pp

Norn M S & Opauszki A (1977) Effects of ophthalmic vehicles on the s[illegible] precorneal film *Acta ophthal (Kbh) 55* 23–34

Author's address

Mogens Norn Eye Department
Hvidovre Hospital DK 26[illegible]0 Hvidovre Denmark

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo, Norway 13–16 June 1979

University Eye Clinic
(Heads V Dreyer J Edmund E Gregersen S Kessing H H Seedorff)
Rigshospitalet Copenhagen Denmark

QUANTITATIVE MEASUREMENTS OF THE FLUORESCENCE IN LIMBAL VESSELS BY DYNAMIC TELEVISION ANGIOGRAPHY

BY

ERIK SCHERFIG JENS EDMUND and
HENRIK LUND ANDERSEN

A method for dynamic angiography and quantitative measurement of fluorescence in ocular vessels in an *in vivo* experiment and in corneo-conjunctival angiography is described

Key words fluorescein angiography – dynamic angiography – fluorescence measurement – television angiography

television exposure is at a rate of 50 images a second and fluorescein angiography performed with TV equipment may be recorded on a tape for later measurement

In angiography the intensity of the light emitted from the fluorescein depends on the amount of fluorescein in the vessels This means that measurment of the light emission from the vessels at different times of the angiography affords a quantitative expression of differences in the time at onset of vascular filling differences in the rate of filling and an expression of the magnitude of oozing

Method

For the television recording of fluorescein angiography we ha e black white camera having the following specifications

Type of camera
2/3 inch Chalnicon electrostatic focusing electromagnetic distortion

Scanning system 625 lines 25 exposures/sec
2 1 interface
Horizontal frequency 15625 kHz
Vertical frequency 50 Hz

Linear distortion
1% at 100% utilization of the camera tube

Geometric distortion 2%

Zoom
100 70 50 40% of the total camera tube

Resolution capability 450 lines

Sensitivity 1 lux

Automatic sensitivity ... by the aid of an automatic lens

Video output
1 volt sync negative ...

Signal to noise ratio Better than ...

Lenses Standard 16 mm ...

Power requirement
100 127 ... 0 240 V 50 Hz ...

Power consumption 14 W

In dynamic angiography of the anterior chamber the television ... nected to one ocular of a slit lamp Through the other ocular the exam... on the areas to be investigated Ten ml 5% sodium fluorescein was ... intravenously The light intensity in the slit lamp was not altered

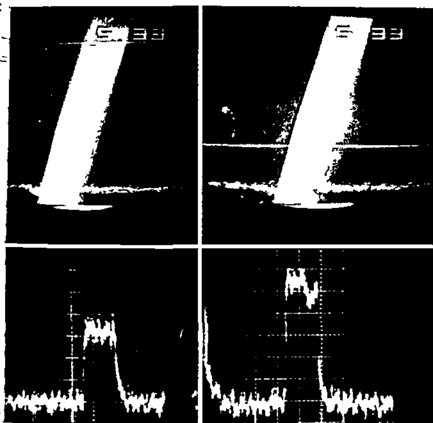
Simultaneously with the TV angiography the numbering of each TV ... was recorded

Measuring method

In vitro investigation A plastic tube is filled with a solution of fluorescein and placed in front of the slit lamp The television exposure is started and pure water is ... anteriorly into the tube This causes dilution of the fluorescein manifesting ... a decrease in ligh intensity on the TV image and a corresponding decrease ... voltage of the video signal recorded on the video tape The rate at which the decrease in voltage takes place expresses the flow of the injected water

On each TV image whose number has been recorded simultaneously with the exposure two points are selected at a distance of 10 mm on the plastic tube (Fig 1 and 2) The voltage of the video signal in the line where the point is situated ... TV image is demonstrated on an oscilloscope (Figs 3 and 4) and the voltage is measured as the height of the excursion on the curve The width of the plastic tube is seen as the width of the voltage increase on the oscilloscope curve

By measuring on image by image a diagram is plotted (Fig 5) on which one curve represents the change in fluorescence at one point and the other curve ...



Figs 1—4

1 Still of TV image No 533 with recording of the line at which the light intensity is to be asured.

2 Still of TV image No 533 with record ng of the other line at which the light intensity to be measured

3 Recording on the oscilloscope of the voltage of the video signal through the line in the a e in Fig 1

4 Recording on the oscilloscope of the oltage of the deo s gnal through the line on mage in Fig 2

ange in fluorescence at the other point. On the diagram in Fig 5 the decrease in uorescence – measured as the fall in the voltage of the video signal – is parallel for e two points but chronologically shifted The chronological shift is 0 08 sec, corresponding to four images At the selected distance of 10 mm between the points e linear rate is 12 5 cm/sec At the diameter of plastic tubing used the volume flow about 0 5 ml sec

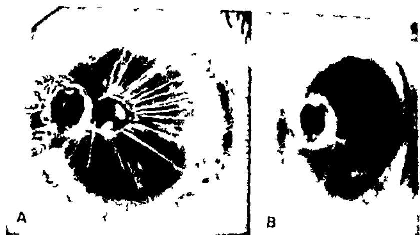


Fig 3

Corneal angiogram in descemetocele showing (A) small regressive pannus all around descemetocele blood vessels with (B) diffuse leaka e in late phase



Fig 4

Angiogram in chronic corneal ulcer showing (A) progressive pannus in surg per e cornea all around limbus and (B) in late phase negati e shado of the cornea surrounding fluorescence corneal ulcer stained with fluorescein (arrow) and a fluorescence of iris

The iris vessels showed fluorescein leakage The corneal ulcer was not seen in early -ein angiograms but in the late phase it showed intensive fluorescence (Fig 1B) After -neal ulcer had totally healed the corneal macula did not show any marked ence in the late phase

t marginal ulcer

ear-old male had a simple marginal catarrhal ulcer nasally in the left eye In the tein angiograms adjacent to the ulcer congested conjunctival vessels and dilated capillaries were seen as an early microscopic pannus showing fluorescein leakage and pearance of negative shadows in the late phase Fluorescein leaked from the limbal nto the ulcer which showed increasing fluorescence in the late phase

-metocele

ear-old woman was admitted with descemetocele small fistulous perforation and or synechia of the iris in the right eye which had been treated with local antib otics. In



Fig 5

-ogram in corneal leucoma with lipid keratopathy showing pronounced fluorescence in area of l p d deposition

fluorescein angiograms the conjunctival and iris arteries filled [illegible] small regressive pannus all around the limbus (Fig 3A) Blood vessels [illegible] leakage in the late phase surrounded the descemetocele (Fig 3A,B).

Chronic corneal ulcer

A 65 year-old male had chronic corneal ulcer in the left eye Fluorescein a[illegible] progressive pannus invading the peripheral cornea around the [illegible] (Fig [illegible]) fluorescein leakage from the new vessels which appeared as negative [illegible] surrounding fluorescence in the late phase (Fig 4B) The iris vessels showed early [illegible] leakage The corneal ulcer stained with fluorescein in the late phase

Corneal leucoma with lipid keratopathy

A 84 year old woman with a history of a corneal ulcer over 10 years earlier was [illegible] vascularized leucoma showing lipid deposition in its superior part [illegible] fluorescein angiograms narrow radial straight arteries were seen to extend [illegible] cornea where they formed a capillary plexus this showed early mottled fluorescence [illegible]

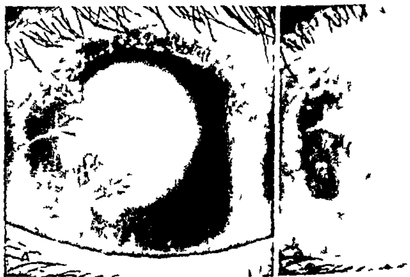
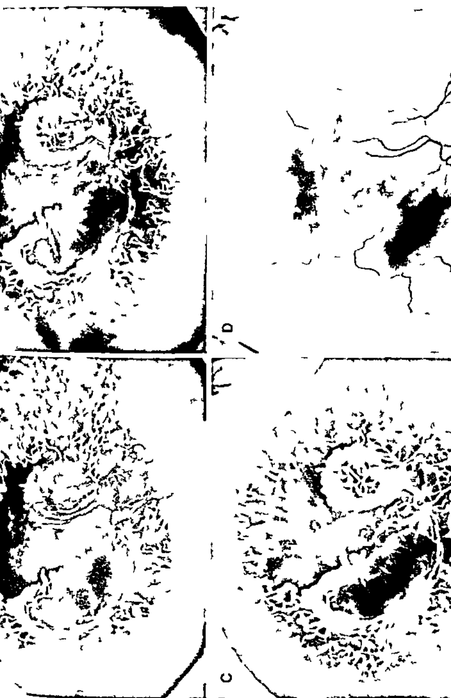


Fig 6

Angiogram in disciform keratitis showing (A) filling of limbal vessels [illegible] and early leakage from apex of the vessels and (B) in venous phase [illegible] corneal vessels and peripupillary leakage in the iris

Fig 7

Corneal angiogram in rosacea keratitis (A) Filling of limbal pannus [illegible] extending into central cornea (B) Network of vascular [illegible] areas with early leakage (C) Venous phase [illegible] profuse fluorescence [illegible] of some circular veins (D) Late phase with [illegible] vessels as negative [illegible] diffuse fluorescence


C
D

which was more pronounced in the area of the lipid deposition (Fig 5 [illegible] sluggish filling and were seen as negative shadows in surrounding fl[illegible] phase

Disciform keratitis

A 54 year-old male had disciform keratitis in the left eye Fluorescein [illegible] progressive pannus with some small vessels loops invading the cornea [illegible] limbus and at the site of the discoid opacity a deep brushlike bundle [illegible] bundles with narrow and straight supplying arteries and arborescent [illegible] leakage was seen from the apex of the new vessels (Fig 6A) The [illegible] fluorescein leakage (Fig 6B) In the late phase the corneal vessels were seen [illegible] shadows in surrounding fluorescence

A 28 year-old female had disciform herpes simplex keratitis [illegible] cornea Fluorescein angiograms showed two narrow and straight deep [illegible] brush like branching invading the cornea at 5 o clock. These deep vessels [illegible] time as the iris vessels whereas a bundle of superficial arborescent blood [illegible] limbal plexus at 4 o clock filled later Both bundles showed diffuse leakage [illegible] the vessels and the discoid oedematous keratitis area stained with fluorescein [illegible] phase

Rosacea keratitis

A 60 year-old female had rosacea keratitis in the mid peripheral areas of [illegible] Anterior segment fluorescein angiograms of the left eye showed a pr[illegible] dilated vessels invading the cornea all round the limbus Arborescent branch[illegible] the end of some vessels Fluorescein leakage was most conspicuous at the apex of [illegible] Some draining veins showed a circular course In the late phase diffuse fluoresc[illegible] largest vessels as negative shadows was seen in the pannus area.



Fig 8 Simultaneous bilateral anterior segment an[illegible]gram in bilateral scler[illegible] pannus in inferior cornea with discrete leakage and peripupillary leak[illegible] (A) and left (B) eye

9-year-old male had bilateral rosacea keratitis with advanced changes in the right
. Fluorescein angiograms of the right eye showed progressive pannus with bending
les extending into the cornea all round the limbus (Fig 7A) A network of vascular
in the corneal periphery and a heavy plexus in the scar areas with early fluorescein
e from the apex of the vessel loops were observed (Fig 7B) In the venous phase the
areas showed pronounced fluorescence (Fig 7C) In the late phase the coarse vessels
seen as negative shadows in surrounding diffuse fluorescence (Fig 7D)

okeratouveitis

year-old woman had sclerokeratouveitis in both eyes with sluggish conjunctival
ation corneal involvement anterior uveitis and in the right eye vitritis and cystic
lar oedema Simultaneous bilateral fluorescein angiograms of the anterior eyes showed
nus in the inferior perilimbal areas with discrete leakage from the tip of the vessels and
upillary leakage of the iris vessels (Fig 8)

r in rheumatoid arthritis

-year-old woman with seropositive rheumatoid arthritis keratoconjunctivitis sicca and a
ry of a cataract extraction in the right eye eight years previously was admitted with a
r formation in the perilimbal area of the right cornea superiorl In fluorescein
ograms the adjacent conjunctiva showed narrow arteries and congestion of the conjunc
capillaries and veins A superficial pannus extended into the gutter as tortuous vessels
discrete leakage

oren s ulcer

r cataract extraction a 63 year-old male developed Mooren s ulcer which spread from
operation scar over the whole cornea in 1 year and 9 months Fluorescein angiograms
months after cataract extraction showed narrow and straight conjunctival and thick
ding episcleral arteries supplying the ulcus area which was invaded by congested limbal



Fig 9

...ram in Mooren s ulcer (A) Congested limbal arcades with earl leakage in ulcer area
(B) Pr fuse leakage from limbal arcades and from iris vessel

arcades (Fig 9A) These arcades as well as the iris vessels showed each pr... limbal veins showed marked congestion with sluggish filling (F... 9B) ... ulcus area and the iris showed diffuse fluorescence

Corneal graft vascularization

A 36 year-old male developed leucoma in the left cornea following a ... Ten years after the accident a penetrating graft was performed through ... graft Angiogram 20 days after the penetrating graft showed a vascular ... into the lamellar graft and a few deep vessels extending to the penetrating junction where they showed a circular course with some vessel ... penetrating graft Diffuse leakage was seen in the penetrating graft interface and ... the vessels in the graft itself

A 29 year-old male was grafted due to vascularized leucoma following a ... the right eye two years earlier He developed vascularized graft rejection, ... twice with 700 rad β irradiation Angiograms five months after corneal ... showed heavy congestion of conjunctival and perilimbal vessels and extension of ... into the periphery of the graft with profuse leakage The iris vessels ... fluorescein leakage (Fig 10)

Complicated acute keratoconus

A 23 year old male with Down s syndrome developed acute keratoconus in the ... was complicated by staphylococcus aureus ulcer Angiograms one month ... disease showed congestion of the conjunctival and limbal vessels with ... progressive pannus into the cornea all around the limbus (Fig 11A) The ... fluorescein leakage from the apex of the vessels which were seen as ... surrounding fluorescence in the late phase (Fig 11B) The corneal ulcer healed ... oedema decreased during vascularization of the cornea



Fig 10
Angiogram of eye grafted for leucoma following tear gas injury shows (A) extension of vessels into periphery of graft (B) profuse leakage from new formed vessels and leakage in iris



Fig 11

ogram in acute keratoconus with staphylococcus aureus ulcer (A) Extension of limbal les into the cornea with leakage at the apex of the vessels (B) Late phase with peripheral eal vessels as negative shadows in surrounding fluorescence and accumulation of fluorescein in margins of central oedematous cornea

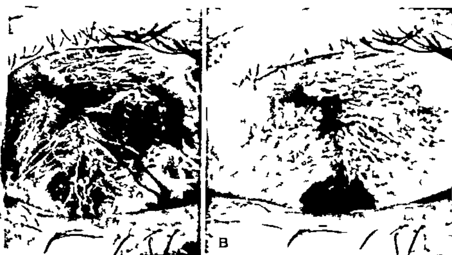


Fig 12

ngiogram in multiple pterygia showing (A) filling of straight arteries and branching vessel ps, and (B) in venous phase extensive network of capillaries and draining veins with discrete leakage

arcades (Fig. 9A). These arcades as well as the iris vessels showed early ... limbal veins showed marked congestion with sluggish filling (Fig. 9B). ... ulcus area and the iris showed diffuse fluorescence.

Corneal graft vascularization

A 36 year old male developed leucoma in the left cornea following a ... Ten years after the accident a penetrating graft was performed through ... graft. Angiogram 20 days after the penetrating graft showed a vascular ... into the lamellar graft and a few deep vessels extending to the ... junction, where they showed a circular course with some vessel ... penetrating graft. Diffuse leakage was seen in the penetrating graft interface ... the vessels in the graft itself.

A 29 year-old male was grafted due to vascularized leucoma following ... the right eye two years earlier. He developed vascularized graft rejection, twice with 700 rad β irradiation. Angiograms five months after ... showed heavy congestion of conjunctival and perilimbal vessels and extension ... into the periphery of the graft with profuse leakage. The iris vessels showed ... fluorescein leakage (Fig. 10).

Complicated acute keratoconus

A 23 year-old male with Down's syndrome developed acute keratoconus in the ... was complicated by staphylococcus aureus ulcer. Angiograms one month after ... disease showed congestion of the conjunctival and limbal vessels with ... progressive pannus into the cornea all around the limbus (Fig. 11A). The ... fluorescein leakage from the apex of the vessels which were seen as ... surrounding fluorescence in the late phase (Fig. 11B). The corneal ulcer ... oedema decreased during vascularization of the cornea.



Fig. 10

Angiogram of eye grafted for leucoma following tear gas injury shows (A) ... vessels into periphery of graft. (B) profuse leakage from new formed vessels ... leakage in iris.



Fig 11

...gram in acute keratoconus with staphylococcus aureus ulcer (A) Extension of limbal ...es into the cornea with leakage at the apex of the vessels (B) Late phase with peripheral ...al vessels as negative shadows in surrounding fluorescence and accumulation of fluorescein in margins of central oedematous cornea.

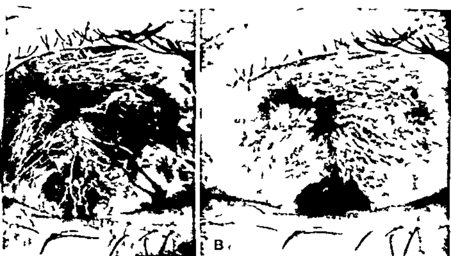


Fig 12

...ogram in multiple pterygia showing (A) filling of straight arteries and branching vessel ...ps and (B) in venous phase extensive network of capillaries and draining veins with discrete leakage

Multiple pterygia with keratoconjunctivitis

A 50 year-old woman with bilateral multiple pterygia and staphylococcal [illegible] junctivitis five days after frequent instillation of antibiotics showed, in fluorescein angio[illegible] grams straight arteries extending from the conjunctiva onto the cornea and [illegible] network of capillaries which was drained by thicker veins (Fig 1). In the [illegible] vessel trunks appeared as negative shadows in diffuse fluorescence

Discussion

This is the first study to show the staining of the avascular corneal [illegible] fluorescein in the late phase of angiography Simple marginal ulcer [illegible] stain from the adjacent limbal vessels which leaked fluorescein The [illegible] might also stain from the limbal vessels in the late phase because [illegible] normal limbus (Mitsui et al 1969) three min after the injection diffuse [illegible] was approaching the centre of the cornea The possibility of fluorescein [illegible] from conjunctival vessels via the tear film to the ulcer could not be [illegible] angiograms

Any vessels in a previously normal cornea are due to neovasculariz[illegible] study in acute complicated keratoconus and in Mooren's ulcer the cornea[illegible] decreased during vascuralization and in corneal graft failures the vas[illegible] followed the immune response to the graft suggesting that the neovascu[illegible] not the cause but the consequence of the corneal inflammatory [illegible] leakage of corneal vessels is a function of the degree of surrounding [illegible] compactness and may serve as an indicator of the amount of [illegible] underlying corneal disease (Kottow 1978) In this study in simple [illegible] marginal corneal ulcers the adjacent limbus showed an early microscopic [illegible] In a chronic corneal ulcer disciform keratitis rosacea keratitis and Moor[illegible] a progressive pannus with profuse leakage was seen The tendency [illegible] fluorescein extravasation was much less in the pannus of sclerokeratou[illegible] gutter in rheumatoid arthritis as was seen by Kottow (1978) in scleroke[illegible] in sclerouveitis

Fluorescein angiograms showed fluorescein leakage of the iris vessels [illegible] irritative iritis during the active stage of simple and chronic corneal ulcer disciform keratitis Mooren's ulcer and in graft rejection In the case of keratouveitis iritis vitritis and cystic macular oedema were seen as [illegible] general ocular inflammation associated with the corneal neovasculariz[illegible]

Acknowledgments

I should like to thank Mr Heikki Nieminen the photographer (Oulu [illegible]) for the photograph

References

~ ric P & Rebiere P (1971) Nouvelles indications de l angiographie fluoresceinique du
~ ment anterieur de l oeil Quelques exemples de pathologie sclerale et corneenne A n
th t (Paris) 204 731–739
dler J W Sewell J H & Kaufman H E (19 5) Anterior segment fluorescein
nography a simple modification of the Zei s stereo slit lamp camera A r Ophthal 7
99
D L & Bron A J (1971) Fluorescein angiography of the anterior segment Its value in
neal disease Brit J Ophthal 55 671–C82
M H (1978) A t r gme t fl escein angi graphy pp 89–98 Williams & Wilkin
ltimore
ra Y Matsubara M & Kanagawa M (1969) Fluorescence irido-corneal photography
t J Ophthal 53 505–512

r all s
atti Saari M D Institute of Clinical Sciences
ersity of Tampere Teiskontie 35 SF 33520 Tampere 52 Finland

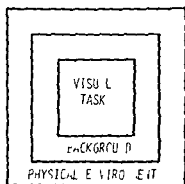
Visual Requirements in Industry

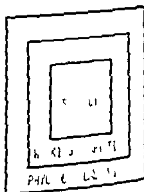
During the last twenty years much effort has been concentrated on the [illegible] ation of visual requirements for workers in various branches of [illegible] Wirt (1945) were the first to study visual performance in relation to job [illegible] mance In a large scale study they obtained statistical evidence to support [illegible] visual acuity for the successful performance of different industrial jobs [illegible] and administrative work productivity fell when the corrected visual acuity [illegible] than 20/40 when the corrected acuity at near was less than 20/30 and [illegible] muscle balance for near was abnormal No influence could be detected [illegible] heterophoria at distance or of colour perception Transport workers [illegible] were found to be more dependent on distant visual acuity on [illegible] perception and on unimpaired bonocular vision at distance Unskilled [illegible] were successful performers even when their corrected distant acuity was [illegible] Muscle balance abd colour recognition were of no importance In the [illegible] such standards are use a guidance when pre-employment eye exam [illegible] practiced to place prospective workers in suitable jobs

After all such standards for fitness in industry are difficult to [illegible] troublesome to collect data from a sufficient number of uniform work [illegible] and as a matter of fact rather few persons have substandard visual capacity

Thus apparent correlation could be inconclusive because [illegible] homogenous observations

Execution of work is dependent on an interaction between the worker [illegible] work (Fig 1) The study of the job itself comprises a classification of [illegible] gross work taking in consideration the critical details and the visual [illegible] available Further qualities to be considered when the background of the [illegible] piece is to be described are the individual working place illumination with [illegible] to both quality and quantity of light glare daylight factor and free view [illegible]





Furthermore the effect of noise heat and humidity must be taken into ınt. Of course the visual performance profile of the employee is mandatory to ısual capacity but other important factors are the working posture and the ıological stimulation i e the relationship between skill and task. Maladjustment ork may cause stress and so may bad co-operation with fellow workers and lload

ıus a job analysis is a complex matter and the influence of one sole factor e g l capacity on productivity security or individual well beeing can only be ızed if all other conditions mentioned are comparable and that is an unusual t. Productivity studies are further jeopardized by the fact that modern luction is often performed in groups with a shifting job routine Thus an idual effort is difficult to evaluate

Present Study

paper only summarize some results from a more comprehensive report lished elsewhere (Clausen et al 1978) The electronic industry concerns solding inting print plates work repair and control and many of these processes esent demanding visual tasks This industry employs both younger and elderly ons and may be considered amble for the study of visual problems The field y was conducted by one academic engineer trained in ergonomics one therapeut, one academic lighting engineer one psychologist and one orthoptist. visual test took place in a mobile specially equipped cabin placed near the kshop

he study involved 162 employees and age varied between 18 and 60 years The age was 39 years Two-thirds were women Several cross references were made n attempt to show relation between observations recorded by the various miners The total amount of persons and the heterogeneous data composition ever invalidated many statistical evaluations Thus the study must be con red a pilot study suggesting tendencies to be proved in studies on a larger scale he tests for visual acuity and for near vision revealed few facts of importance 3% of the subjects had a corrected visual acuity og 6/6 and 97% could read a point print at a distance of 40 cm This leads to the conclusion that a screening hese basic visual functions would have been of limited value in this plant

One could imagine that visual unfit workers had left the industry spontaneously er a short period Such a natural selection could not be demonstrated 38% had uncorrected visual acuity below 6/6 a figure that agrees with the results of the rementioned screening tests 29% of 162 had heterophorias exceeding +1 pd d −? pd read on Maddox Wing test Nor does this figure indicate any selection

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

Department of Ophthalmology
(Heads: F. Goldschmidt, S. Fourschou and A. [illegible])
University Hospital, Odense, Denmark

PRESENILE CATARACT

A follow up investigation with special reference to occupational aspects

BY

DANUTA MARUSZCZAK, HANNE JENSEN
and ERNST GOLDSCHMIDT

A retrospective investigation was carried out of 100 patients below the age of 60 years operated for presenile cataract.
The material has been divided into three occupational groups depending on the visual requirements.
The complaints prior to and after operation have been related to the patients' ability to retain their employment. Of [illegible]8 patients who were still employed only 45 were able to return to their former occupation after the first cataract extraction.
The final result was that 62 patients could continue their former work, [illegible] changed their occupation due to aphakia problems, while four had to retire.
The vision in the fellow eye, the type of work and the optical appliances have been found to be of primary importance.
It is concluded that operation for unilateral cataract is inadvisable if the vision of the fellow eye is good and contact lenses cannot be used, that the [illegible] between operations for bilateral cataract should be as short as possible and that the use of contact lenses is essential.

Key words: presenile cataract – occupational aspect – correction of aphakia – simultaneous bilateral cataract extraction.

g advice to patients suffering from presenile cataract in respect of the choice ... time of operation is difficult. In cases of occupational problems the ...almologist will often be subject to a certain amount of pressure from the ...nt. It must therefore be of interest to determine whether early operation ...mplies with the expectations of the patient, b) permits retention of the ...pation and c) whether the operative method and various forms of correction ...e aphakia influence the results

Material

...ng the period 1 4 1972 to 31 12 1977 143 patients below the age of 60 years ... referred to the Ophthalmological Department of the Odense University ...pital for operation for presenile cataract All those patients were recalled to the ...rtment in the latter part of 1978 for a follow up examination Forty three did ...attend of these 9 were dead 13 had moved to other parts of the country 10 ... in poor health and 11 did not react to repeated inquiries

...he final material thus consists of 100 patients 65 men and 35 women the age ...sex distribution illustrated Table I The average age of the men was 54 5 years ...of the women 53 years

...orty four men and 23 women were operated on both eyes the remaining 33 ...ents on one eye only Thus 167 operations are included in the material

Methods

...ormation was obtained from the case records regarding preoperative visual ...ity operative method as well as complications during admission

Table I
Age and sex distribution of 100 patients operated for presenile cararact

Age (years)	Male	Female
31–40	1	3
41–50	9	7
51–60	55	25
Total	65	35

Table II

Duration of interval between operations in 6 cases of binocular cataract extraction.

Duration (months)	Male	Female
1	2	1
2–6	9	3
7–12	10	9
13–24	12	5
25–36	7	3
37–48	[illegible]	1
49–60	[illegible]	1
Total	44	[illegible]

The follow up examination included the following

1 An interview with the patient regarding the occupational [illegible] length of sick leave

2 Information regarding the correction of the aphakia this was [illegible] patients optician

3 A physical examination including visual acuity biomicroscopy [illegible] ophthalmoscopy evaluation of binocular function by means of Worth's [illegible] and titmus stereo test

The follow up examination was carried out 6–72 months after the [illegible] eye one with an average observation time of approximately 3 years

Table II shows the number of months between the operations in [illegible] binocular operation Only one patient was subjected to operation on [illegible] during the same admission

The preoperative visual acuity in the better eye is shown in Table III [illegible] appears that 50% had a visual acuity of 6/12 or more at the time of operation

Table III

Preoperative visual acuity in fellow eye in 100 patients

Visual acuity	Male	Female	Total
6/6	14	4	[illegible]
6/9–6/12	23	9	[illegible]
6/18–6/24	19	13	[illegible]
6/36	9	3	[illegible]

itive method

–ves were subjected to intracapsular operation with corneo-scleral incision and –peripheral iridectomies the lens was extracted employing the cryotechnique 3 cases enzymatic zonulolysis was used The extracapsular operative technique chosen in 29 cases The average period of hospitalization was 10 days for ation on eye one and 8 days for operation on eye two

Results

al improvement is shown in Table IV The preoperative visual acuity of the 167 ated eyes is related to that immediately postoperatively (approximately 8 days) the vision at the time of follow up As expected a pronounced improvement in visual acuity is seen and 90% had ≧ 6/12, without any significant difference een intra and extracapsular operation However a vision of 6 6 is seen ificantly more frequently in those subjected to intracapsular operation

he operative and postoperative complications are shown in Table V The most uent complication was transient ocular hypertension from day 2 to 6 post ratively It was necessary to re admit 8 of 29 patients subjected to extracapsular ration for discission However this was not required in any of the seven cases i capsular rupture

t the time of the follow up examination the vision was < 6/18 in 12 (8 7%) of eyes operated intracapsularly and in four (13 8%) of the extracapsularly rated eyes The causes of the reduction in vision are shown in Table VI

Table IV
Visual results in 167 eyes

Visual acuity	Preoperative	Postoperative	
		8th day	Follow up
Intracapsular extraction			
6 6		41	102
6 9 –6/12	1	58	24
6 18–6/24	15	31	4
6 36	122	8	8
Extracapsular extraction			
6 6		8	15
6 9 –6/12		6	10
6 18–6/24	4	8	1
6 36	25		3

Table V

Operative and postoperative complications in [illegible] eyes

Complication	Intracapsular extraction		[illegible]	
	Male (92 eyes)	Female (46 eyes)	Male ([illegible] eyes)	Female ([illegible] eyes)
Broken vitreous	1	1		
Ruptured capsule	5	2		
Ocular hypertension (>25 mmHg in 2–6 days)	31	9	5	1
Hyphaema	2	1		
Vitreous haemorrhage	1	1		
Cystoid oedema	2			1
Retinal detachment	5			1

Occupational aspects

An attempt has been made to evaluate the occupational aspects, both pre[illegible] postoperatively

The material has been divided into three occupational groups, depe[illegible] visual requirements The distribution is shown in Table VII

The results of the investigation are shown in Tables VIII and IX a dis[illegible] being made between those operated on one eye only and those subjected to [illegible] operation

Table VI

Ocular pathology in 1[illegible] intra and 4 extracapsular operated eyes with ≤6 18 visual acuity at the time of follow up examination

Finding	Intracapsular extraction n = 1[illegible]3	Extracapsular extraction n = [illegible]
Vitreous haemorrhage	2	–
Secondary cataract	1	2
Retinal detachment	3	–
Cystoid oedema	1	1
Corneal opacity	[illegible]	1
Diabetic retinopathy	2	–
Amblyopia	1	–
Total	1[illegible]	4

Table VII
Occupational activities in 100 patients

	Occupational category	Male		Female	
		Unilateral aphakia	*Bilateral* aphakia	*Unilateral* aphakia	*Bilateral* aphakia
	1 High visual* requirements	6	19	5	3
oved rs	2 Moderate visual** requirements	4	9	2	8
	3 Close work***	9	12		1
fe 4 ed				4	10
		2	4	1	1

Skilled workers with fine work drivers ships officer etc
Skilled and unskilled workers with heavier work farm labourers etc
Executive teaching or office work

Table VIII
Occupational and sick leave in 26 patients operated for unilateral cataract

	Occupational category						Total n = 26
	1		2		3		
	Male n = 5	Female n = 6	Male n = 4	Female n = 2	Male n = 9	Female	
reoperative							
Sick leave	–	3	–	1	1	–	5
Change of occupation	1	–	–	–	–	–	1
Severe difficulties	5	2	4	1	8	–	20
No difficulties	–	–	–	–	–	–	–
Postoperative							
Sick leave	–	1	–	–	–	–	1
Change of occupation	–	1	1	1	–	–	3
Severe difficulties	–	–	–	–	–	–	–
No difficulties	6	3	3	1	9	–	22

Table IX
Occupational and sick leave in 52 patients operated for bilateral cataract

	Occupational category					
	1		2		3	
	Male n = 19	Female n = 3	Male n = 9	Female n = 8	Male n = 1?	Female n 1
Preoperative						
Sick leave	8	1	1	3	1	
Change of occupation	3	–	2	1	–	
Severe difficulties	8	2	6	4	11	1
No difficulties	–	–	–	–	–	
After 1st operation						
Sick leave	3	–	–	–	1	1
Change of occupation	3	1	3	1	1	
Severe difficulties	7	1	1	–		–
No difficulties	2	1		7	8	
After 2nd operation						
Sick leave	1	–	–	–	1	1
Change of occupation	3	–	2	1	1	
Severe difficulties	–	–	–	–	–	
No difficulties	13	3	7	7	10	–

Preoperatively all 78 had difficulty in carrying out their work, and was [illegible] contributory factor in the desire for operation Seven of the 78 had [illegible] wholely changed occupation and 19 had been on sick leave for up to [illegible] prior to admission

Postoperatively i e after operation on eye one there were [illegible] depending on the vision in the fellow eye the type of work and the [illegible] appliances Only 45 of the 78 patients were able to return to their work without special problems after the usual period of convalescense

A more thorough analysis showed that there is a relationship between the [illegible] in the unoperated eye and the working ability postoperatively

Of 26 patients (Table VIII) operated for unilateral cataract [illegible] return to their work after operation The majority had good visual acuity in the fellow eye and as it is shown later accepted contact lenses Among [illegible] operated for bilateral cataract (Table IX) only 23 could after operation [illegible] eye continue in their former work without difficulties

number of patients despite a successful operation on the first eye reuquired a er period of sick leave or were forced to change their occupation This applies articular to patients in occupation This applies in particular to patients in pational group 1

ie 5 men in occupational group 1 (Table IX) who required postoperatively sick (9 to 6 months) had poor vision in the fellow eye (≦6/24) The period of time r to operation of the fellow eye was from 2 to 9 months

ve men and one woman in the same group 1 changed their occupations after a essful operation on eye one as they were unable to carry on work without cular function In these cases the vision in the fellow eye was also relatively r (≦6/18) Operation on the second eye was not carried out until after 6 to 36 nths

1 group 9 where the requirements to vision were less exacting no patient ded sick leave between the operations but 3 men and 1 woman changed their upation due to the aphakia problems the vision in the fellow eye was also ≦6/18 ee patients waited between 10 and 24 months for operation on the second eye

n group 3 3 patients who had to have sick leave or change their work also had r vision in the fellow eye The situation changed considerably after the ration on eye two (cf Table IX bottom)

he final result was that 62 patiens from the total group of 78 stil employed ents could continue in their former occupation 12 changed their work due to akia problems while 4 had to retire

The housewives with bilateral cataract had severe problems after operation of eye and needed assistance in housekeeping After the second operation no istance was necessary

Table X

Glasses or contact lens wear related to visual acuity of phakic eye in 100 pat ents

Visual acuity in fellow eye	Male			Female		
	No of patients	Glasses	Contact lens	No of patients	Glasses	Contact lens
6 6	14	2	19	4	1	3
6 9 –6 12	23	5	17	9	6	3
6 18–6 24	19	13	4	13	8	4
≦6 36	9	8	1	9	8	1
T tal	65	28	34	35	23	11

Three men and one woman had no correction for the aphakia

Table XI
Contact lens wear related to age in 100 patients

Age (years)	Male		Female	
	No of patients	Contact lens	No of patients	Contact lens
31–40	1	1	5	1
41–50	9	5	[illegible]	3
51–60	55	28	25	5
Total	65	34	35	11

A number of patients were only able to retain their occupation [illegible] were permitted to drive a vehicle Of 61 patients with driving licence [illegible] previously driven a car 31 stopped driving prior to the operation Of these [illegible] resumed driving after operation

Selection of correction for aphakia

Table X shows the correction for the aphakia as related to the vision in the [illegible] eye Four patients had no correction for the aphakia and many were first given correction 3–6 months after operation It can be seen from the table that 34 (52 3%) and 11 women (31 5%) had contact lenses but of these 5 women had to discontinue the use mainly due to irritation and difficulty in the insertion [illegible]

There is a relation between visual acuity on fellow eye and choice of correction [illegible] aphakia Of the 50 patients operated for unilateral cataract with 6/6–6/12 [illegible] fellow eye 70% employ contact lenses

In the other group of 50 patients with visual acuity worse than [illegible] unoperated eye only 20% wear contact lenses

The contact lens frequency is somewhat lower in women but among those [illegible] an occupation the frequency is almost as high as in men

Table XII
Contact lens wear related to occupational category

Occupational category	No of patients	Contact lens	%
1	33	21	64
2	25	5	20
3	22	14	64

ourteen patients with glasses and visual acuity 6/6–6/12 in the fellow eye had ost no benefit from the operation and the majority used the phakic eye operatively

he age distribution of the patients who primarily employed contact lenses is wn in Table XI The motivation for employing contact lenses appears to be ewhat stronger in men than in women and in younger patients Table XII ws the use of contact lenses in the various occupational groups and it may be that patients with high visual requirement or involving much close work use tact lenses far more frequently than patients with moderate visual requirements

ocular function

binocular function was studied by means of the Worth 4 DOT test and the ius stereo test The results are shown in Table XIII from which it can be seen t the binocular function was considerably better in those employing contact ses than in those using spectacles

Table XIII

Stereo-acuity in 19 unilateral aphakes wearing contacts and 66 bilateral aphakes wearing contacts or glasses

	Sex	No of patients	Full stereopsis No of patients	%
Unilateral aphakes n = 19				
Contacts	Male	15	7	
	Female	4	3	
	Total	19	10	52 6
Bilateral aphakes n = 66				
Glasses	Male	27	8	
	Female	19	7	
	Total	46	15	32 6
Contacts	Male	16	12	
	Female	4	2	
	Total	20	14	70

Discussion and Conclusion

The present investigation which includes 60 men and 30 women [illegible] 60 years has been carried out retrospectively During the period [illegible] clear lines of direction have been laid down regarding the selection of [illegible] operation and the optimal period between the operations on the two eyes

The investigation shows that the final vision is comparatively [illegible] whether intra or extracapsular technique is employed and that [illegible] postoperative complications Re admission for discission of the [illegible] considered an extra strain by the patients even though the period of [illegible] is short

Occupational problems have been a major factor in the desire for [illegible] number of patients have changed their occupation prior to the operation [illegible] been granted sick leave while some others who have worked [illegible] operation found that this was only possible due to the fact that they [illegible] reduce the speed at which they worked or who had sympathetic [illegible] helpful colleagues

A contributory cause to the postoperative problems is undoubtedly [illegible] the patients expect cataract extraction to solve all their visual problems [illegible] opinion is undoubtedly held by many colleagues at work, employers and [illegible] The long period of waiting between operations had been a further [illegible] waiting time should be avoided In this connection it should be emphasised [illegible] patients comprising the present material have been referred to the department [illegible] 10 different practicing ophthalmologists. After discharge the patients [illegible] consult them these ophthalmologists also determined the type of [illegible] required for the aphakia

Unilateral cataract extraction with a wellpreserved vision in the fellow eye [illegible] not appear to give any greater problems neither pre nor post [illegible] patients adapt themselves to the situation also in respect of their [illegible] the majority are able to return to their previous trade or profession [illegible] period of postoperative sick leave This group of patients seems to be [illegible] inclined to use contact lenses and do not have any greater problems [illegible] including insertion and removal The patients who cannot or will not [illegible] lenses have little or no benefit from the operation and therefore [illegible] deferred as long as possible The group of patients who present with [illegible] cataract development in both eyes are those with the greatest problems [illegible] cataract extraction gives an improvement in vision from which the [illegible] limited beneficial effects Only a few are able to employ contact lenses and [illegible] have serious problems in respect of their occupation particularly [illegible] requiring especially good eye sight The situation does not improve [illegible]

-een performed on the second eye and the investigation clearly demonstrates the interval between operations has been too long The previous assumption operation of the fellow eye should be deferred until the patient has been cribed correction for the aphakia and has become accustomed to the use of this not appear to be reasonable especially for those patients who are still ployed In recent years there has also been an increasing number of ophthal ogists who have advocated the use of simultaneous binoculaar cataract extrac (Jardine 1971 Nissim & David 1977 Suchting 1973)

he risk of postoperative bilateral intraocular infection seems to be overesti ed Jardine performed 585 simultaneous cataract extractions and there were e cases of intraocular infection but one eye only in each case Nissim reported e cases of monolateral endophthalmitis in 676 bilateral cataract extractions in session

nother possibility is to carry out extraction with an interval of a few days during same hospital admission This is advantageous for the patient, as it requires a nsiderably shorter total period of sick leave and is less expensive for the mmunity

The patient should be given preliminary correction for the aphakia a few days er the operation in cases of bilateral cataract extraction carried out during the ne hospital admission

As many patients as possible should employ contact lenses and it is also important at the patients are given correction suitable for their particular work

None of the patients examined had intraocular lens implants but from the perience gained from elderly patients with clip lenses it is obvious that this form correction of the aphakia is superior to any other

References

rdine P (1971) Simultaneous bilateral cataract extraction *T ans ophthal Soc U K 90* 19–,94

ssim J & Robert D (197,) Bilateral cataract extraction in one session report on five years experience *Brit J Ophthal 61* 619–621

chting P (1973) Zur Problematik einer Katarakt Extraktion beider Augen in einer Sitzung *Klin Mbl Au enheilk 163* 9?–96

uth s *dd es*

Maru zzak Department of Ophthalmology
niversity Hospital DK 5000 Odense C Denmark

Results

Industrial measurements

The flux densities and the intervals within which they normally vary at the [illegible] ordinarily occupied by the operator are shown in Figs 1 and 2

The welding processes generated magnetic fields with frequencies of [illegible] and with flux densities within the range $10^{-4}-10^{-7}$T which can be compared [illegible] the earth magnetic field which varies from about 0 03 to about 0 0[illegible] mT Fig 1

Considerable variations may occur due to the current strength [illegible] proximity to metal bodies etc Nevertheless the values given may be [illegible] characteristic of the different methods respectively

Among the electric steel processes the ladle furnace gave a rather high [illegible] density in the proximal zone Particularly high values were recorded [illegible] induction heaters For induction heaters both magnetic flux density and fre[illegible] may be high (up to 10 kHz) implying a high energy density of [illegible] compared to other electrical processes in the frequency range dealt with in [illegible] work (Fig 2)

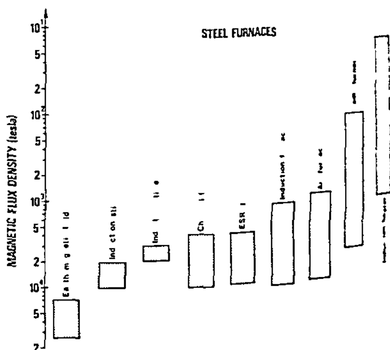


Fig 2

Magnetic flux densities near (at the site of the operator) different steel furnaces [illegible] electroslag refining)

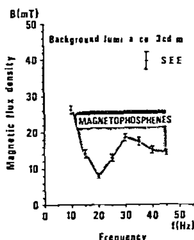


Fig 3

etophosphene threshold curve at a background luminance of 3 cd m² Phosphenes are seen at magnetic flux densities above the threshold curve 10 volunteers

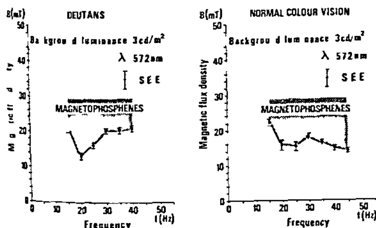


Fig 4

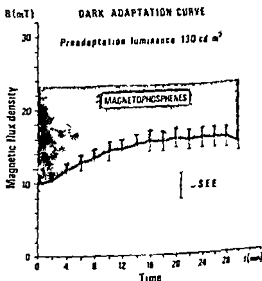
gnetophosphene threshold curve for deutans and volunteers with normal colour vision at 2 nm and 3 cd/m² background light, 9 and 6 volunteers respectively

Magnetophosphenes

The threshold values for magnetophosphenes at the d fferent backgr... nance levels were determined as in Fig 3 in which the magnetic ... values are plotted as a function of the frequency of the magnetic field ... value is defined as the magnetic flux density at which the phosphene ... disappears after having been seen at higher flux densities Every ... each single volunteer is the mean of two determinations

The threshold values varied with the frequency of the magnetic field ... luminance level of the background light. In virtual darkness there was ... maximum at 30 Hz At higher luminances this sensitivity maximum ... towards lower frequencies (20–25 Hz) and a second maximum was ... about 40–45 Hz The maxima were separated by a local sensitivity ... 30–35 Hz The local minimum at 30 Hz was clearly seen at 3 cd/m² ... highest luminance (130 cd/m²) the curve was rather flat above 90 Hz

The thresholds (at 3 cd/m²) for the colour defectives differed from those ... volunteers with normal colour vision The threshold curve for the ... a sensitivity maximum at 20–25 Hz and a local sensitivity minimum at ... Thereafter the sensitivity increased again (Fig 4) The maximum and the ... were stationary at the same frequency also when the wavelength of the ... light was changed The threshold curves for the deutans did not ...



F 5
Threshold values for magnetophosphenes determined during dark adaptation ... the frequencies 20 30 and 35 Hz 8 volunteers

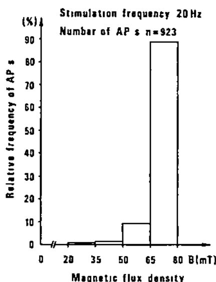


Fig 6

responses in the ganglion cell layer as function of the magnetic flux density at 20 Hz the first second after switching off the field (AP s action potentials)

l sensitivity minimum and the following sensitivity increase for the wavelengths (Fig 4) and 531 nm while they were present at 443 nm

he results of the dark adaptation studies is shown in Fig 5 With increasing time he dark the threshold values also increased

inal ganglion cell activity induced by ELF magnetic fields

e responses from the ganglion cell layer varied to a great extent with the gnetic flux density and the field frequency In accordance with the responses to ht, the responses to magnetic field stimulation occurred only at ON and/or OFF The variation of the responses from OFF-cells with the magnetic flux density the st second after switching off the field is exemplified in Fig 6

Discussion

he purpose of the industrial measurements was to establish the range within hich the magnetic flux density could conceivably vary in different energy nsuming electrical processes in the welding and steel industries With this nowledge it should be possible to simulate corresponding fields under laboratory nditions and also to describe and predict possible physiological effects

The aim of the magnetophosphene studies was to determine ... phosphenes and to shed some light onto the problem of phosphene ... discovered that the electromagnetic fields interact with the visual ... reproducible way ELF fields of moderate flux densities found ... induce magnetophosphenes The relations between the threshold ... magnetophosphenes on one hand and magnetic field frequency ... wavelength of the background light colour vision properties and retinal ... level on the other hand seem to reflect that the phosphenes are generated ... same retinal channels that are normally propagating signals related ... different qualities and that the sensitivity of different channels is related ... frequency of the stimulus We have not found evidence of any ... the visual system of short term exposures to these types of magnetic ... However the results do not permit any definite conclusions as to possible ... long term exposures in welding and electrosteel industries

As an effort to further explain the origin of the magnetophosphenes ... the intraretinal measurements of the electrical activity of the ... results from this part of the study clearly prove that the retina is excited by ... field stimulation They also indicate that the phosphenes are generated in the ... and that there are several similarities between the responses in the ... layer to light and to ELF magnetic fields Possible mechanisms ... magnetophosphenes will be further discussed in a following paper ... 1979)

Our results may be of guidance when formulating threshold limit values ... densities for ELF electromagnetic fields in the industry

Acknowledgments

Miss Birgitta Svensson at the Department of Biomedical Engineering ... is acknowledged for skilful assistance

This work was financially supported by the Swedish Work Environment ... 71/138 77/172) and by the Swedish Medical Research Council (Project No ...

References

D'Arsonval A (1896) Dis positifs pour la mesure des courants ... frequences *Compt rend Soc biol 3* 450–4 ...

Lövsund P Öberg P Å & Nilsson S E G (1977a) Quantitative ... values of magnetophosphenes *1977 International Symposium on the Biological Effects of Electromagnetic Waves* Oct 30 – Nov 4 1977 Airlie Virginia USA p ... publ in Radio Science suppl 1979)

nd P Oberg P Å & Nilsson S E G (1977b) A method for the study of retinal
lion-cell activity induced by ELF magnetic fields *1977 International Symposium on the
gical Effects of Electromagnetic Waves* Oct 30 – Nov 4 1977 Airlie Virginia USA p
Accepted for publ in Radio Science suppl 1979)

nd P Oberg P Å & Nilsson S E C (1978) ELF magnetic fields in electro-steel and
ding industries *Open Symposium on the Bi logical Effects of Electromagnetic Waves held on the General Assembly of the International Union of Radio Sci nce* August 1–8 1978 Helsinki
and p 99

nd P Nilsson S E G Reuter T & Öberg P Å (1979) Magnetophosphenes A
ntitative analysis of thresholds *Med & Biol Eng & Comput* (Accepted for publication)

rs add e ses

osund M Sc and Prof P Åke Oberg Department of Biomedical Engineering
rsity Hospital S 581 85 Linkoping Sweden

Sven Erik G Nilsson Department of Ophthalmology
rsity Hospital S-581 85 Linkoping Sweden

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo, Norway 13–16 June 1979

Department of Ophthalmology (Head: Salmi) ... University of Helsinki, Helsinki, Finland

Short Communication

PERFORATING EYE INJURIES CAUSED BY OCCUPATIONAL ACCIDENTS TREATED AT HELSINKI UNIVERSITY EYE HOSPITAL IN 1970 TO 1977

BY

MATTI NIIRANEN

192 consecutive perforating eye injuries caused by occupational accidents in 1970–77 were studied. They represented 40% of all perforations treated during that period at the Helsinki University Eye Hospital. The results were compared with the previous series from this hospital from 1930–39.
92% of the patients were males, a slight decrease from the fifties. The age group 16–25 years was largest (30%). Previously the next group 26–35 years had been the largest.
The proportion of workers in building construction had increased ... and comprised 26.6%. Another large group were metal workers ...
Agricultural work had lost its importance as the cause of perforations as compared with the previous decades.
The commonest cause of accident was still hammering. Flying chips, especially metal splinters, were important causes in both the present and the previous series.
Prognosis was much better in the present series than earlier ... of patients achieved vision of 0.5 or better, as compared with 40% ... and 20% in the thirties.

Key words: perforating eye injuries – occupational perforations – ... perforations – prognosis of perforations

Author's address: Matti Niiranen, M.D., Tuohikuja 6 D, SF-02150 Espoo 15, Finland.
To be published elsewhere.

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

D partment of Ophthalmology (Head Jon O Ihler)
Telemark Sentralsjukehus Skien Norway

CHLOROQUINE KERATOPATHY IN CHLOROQUINE WORKERS AFTER TOPICAL DUST EXPONATION

A Case Report

BY

LEIF STEENE ERIKSEN

A report of acute corneal changes in workers employed in the chloroquine industry is presented The workers were exposed to chloroquine phosphate dust in the manufacture of chloroquine tablets and the corneal alterations were similar to those described after long continued systemic chloroquine therapy

Key words cornea – chloroquine – keratopathy – dust exponation – man.

loroquine is a quinoline derivative with a coplanar fused ring system It was oduced as an antimalarial drug in 1945 and is today the drug of choice for the atment of malaria Since 1950 it has assumed increasing prominence in the atment of chronic rheumatoid arthritis and lupus erythematosus Long con ued systemic use of this substance is known to produce side effects the most portant of which are ophthalmological Two distinct types of ocular side effects known one affecting the cornea and the other the retina.

Chronic systemic treatment with chloroquine induces morphological alterations the cornea in a proportion of cases Chloroquine is transported by the blood eam to the corneal epithelium via the perilimbal vascular plexus or via the tear nd and tear fluid The epithelial alterations are very characteristic in appearance d fairly constant in localisation The most characteristic features are slightly wavy anulated lines runnning horizontally at the junction of the lower and middle irds of the cornea This fairly constant localisation and peculiar shape is thought

to be due to a form of massaging effect exerted on the epithelium by the [illegible] eyelids

Chloroquine disturbs the normal phospholipid catabolism of the [illegible] thelium and the alterations in the cornea seem to be part of a [illegible] phospholipidosis (Seiler et al 1977) Typical intracellular deposits are [illegible] epithelium cells Similar deposits occur in all tissues with high [illegible] or turnover It has been reported that about 30–45% of the patients [illegible] medication with chloroquine develop these corneal changes (Sandv [illegible] is no direct relationship between the total dose used and the [illegible] differences are presumably due to an individual variation in drug [illegible] corneal changes do not affect the visual acuity they are reversible [illegible] after the treatment is discontinued

Case Report

A 45 year-old man previously employed in a furniture shop [illegible] producing factory The first day in his new job he began to manufacture [illegible] This type of tablets had not been manufactured in the factory for [illegible] the workers had complained of discomfort in the eyes During the production of [illegible] tablets the man was continuously exposed to chloroquine phosphate dust He [illegible] mask to cover his mouth and nose but had no eye protection. Irritation [illegible] present from the first day During the next few days his eyes became red and [illegible] vision became blurred After four days he was referred to the eye department [illegible] punctate erosions of the corneal epithelium were found After three days [illegible] therapy a control examination was performed The epithelium had healed [illegible] changes in the cornea similar to those described after long-continued [illegible] therapy Except for the corneal alterations the entire anatomical and functional [illegible] of the eyes were normal Six weeks later the corneal changes had [illegible] employee was in the same production and also complained of eye [illegible] exposed to the dust to a lesser extent and was not examined in the eye department

Five months later there was a new production of chloroquine tablets [illegible] pollution was reduced and the workers used spectacles to protect their eyes [illegible] spectacles were not used continuously throughout the day Our patient [illegible] red and irritated eyes Also on this occasion he had typical [illegible] examination after five days in the work Two other workers were [illegible] production They had the same symptoms On examination one was found [illegible] chloroquine keratopathy

Discussion

A report has been given of acute corneal changes in workers [illegible] chloroquine industry the changes consisting of deposits in the corneal [illegible] The deposits developed after topical dust exposition and the [illegible]

ar to those described after long-continued systemic chloroquine therapy This iefly of interest from an ergo-ophthalmological point of view Mann (1947) ribed similar corneal changes in quinacrine (Atabrine) workers after local dust ination *Under controlled circumstances essentially the same changes were* roduced in animal eyes by insufflation Side effecets are reported during clinical tment with almost all drugs In the production of potential toxic drugs siderable side effects can be due to direct contact with the eyes It is therefore ortant to protect the eyes and reduce dust exponation

References

in I (1947) Blue haloes in Atabrin workers *Brit J Ophthal 31* 40–46
dik K (1966) Chloroquine effects on the cornea *Acta ophthal 44* 355–360
r K U Thiel H J & Wassermann O (1977) Die Chloroquinkeratopathie als Beispiel ner arzneimittelinduzierten Phospholipidosis *Klin Mbl Augenh ilk 1 0* 64–73

hor s ad lress
f Steene Eriksen Eye Department
emark Sentralsjukehus Betanien Hospital N 3700 Skien Norway

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

The Department of Ophthalmology [illegible]
University of Göteborg Göteborg Sweden

NEW ASPECTS ON THE HEREDITY OF OPEN ANGLE GLAUCOMA

By

TORD JERNDAL and ANDERS L[illegible]D

Open angle glaucoma is known to be inherited but detailed knowledge [illegible] primary genetic factor and its mode of inheritance are lacking. By [illegible] both the glaucomatous and non glaucomatous members in afflicted [illegible] it has been established that premorbid goniodysgenesis inherited in an autosomal dominant mode plays a decisive role for the eventual [illegible] of glaucoma. Goniodysgenesis has been documented not only in [illegible] congenital glaucoma but also in late congenital glaucoma (often [illegible] as glaucoma simplex) and in families with pigmentary glaucoma [illegible] tion glaucoma.
In addition there is evidence that goniodysgenesis is the morphologic prerequisite of the high elevation of the IOP on provocation with [illegible]
The article contains a short survey of the literature and presents three [illegible] with intrafamilial variations of open angle glaucoma based [illegible] genetic trait [illegible] goniodysgenesis.

Key words: genetics – glaucoma – goniodysgenesis – heredity – [illegible] glaucoma – pedigree

It is well known that open angle glaucoma may run in families as an [illegible] disorder. In textbooks on heredity François (1961) and Waardenburg et al. [illegible] is stated that infantile congenital glaucoma (buphthalmus) is recessive [illegible] but juvenile glaucoma and glaucoma simplex are dominant.

In contradistinction Becker (196?) and Armaly (1966) in a [illegible] designed a genetic model which according to their conclusions speak [illegible] recessive mode of inheritance of simple glaucoma.

Unfortunately the entire subject is most unclear due to the lack [illegible] criteria and unequivocal terminology.

o be able to draw valid conclusions from studies of glaucoma pedigrees the owing demands must be met with

The primary genetic trait should be searched and if possible defined

All members glaucomatous as well as non glaucomatous should be properly mined with regard to the genetic trait

Criteria for the diagnosis of glaucoma must be defined

The type of glaucoma should be classified with a careful gonioscopy

these four demands were to be fulfilled approximately 90 per cent of the rature on hereditary glaucoma would be disqualified The remaining 10 per it, however lend themselves to an analysis from which the following conclusions the drawn

Infantile congenital glaucoma

ie important primary point of view is to recognize infantile congenital glaucoma the consequence of goniodysgenesis Andersson (1939) Kluyskens (1950) Worst 966) From this starting point it is no neckbreaking step to assume that infantile ngenital glaucoma is a severe form of dysgenesis of the drainage pathways a ilder form of which may result in juvenile or adult glaucoma

The first pedigree (Fig 1) although small demonstrates this in a clear manner rndal 1968) The dominant trait – goniodysgenesis – is demonstrated in six embers Glaucoma has so far developed in three of them at the age of three onths 10 years and 46 years respectively This pedigree has a strong resemblance that published by Gillespie (1963) with the title Congenital glaucoma juvenile aucoma chronic simple glaucoma all in one family"

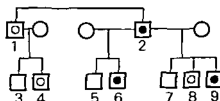
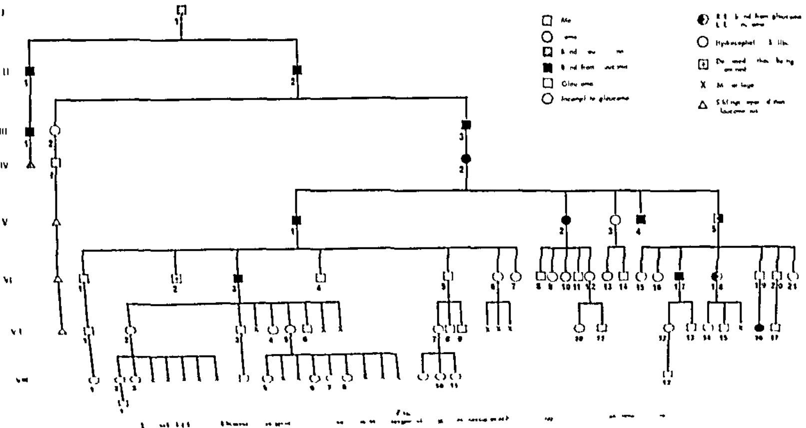


Fig 1

rndal (1968) Member No 9 developed infantile congenital glaucoma at 3 months of age No 6 his half-brother developed late congenital glaucoma at 10 years and their father No developed glaucoma at 46 years They all had distinct goniodysgenesis Moderate goniodysgenesis also found in members 1 4 and 8


I
II
III
IV
V
VI
VII
VIII
Glaucoma
Incomplete glaucoma

Juvenile glaucoma

poorly defined class of glaucoma is characterized by autosomal dominant dity dysgenesis of the anterior ocular segment (Berg 1932 Weatherill & Hart 3) and a poor prognosis on medical therapy

he pedigree reported by Berg was re-examined in 1970 by Jerndal (Fig 2) who ddition to the previously reported kerato- and irido-dysgenetic signs also overed goniodysgenesis in the glaucomatous members There are at least three nbers who developed a verified infantile congenital glaucoma before one year ge the rest of the glaucomas appeared between 1 and 43 years of age

simple glaucoma

een above the same dominant dysgenesis may result in a glaucoma from early ncy to the forties A pedigree from the monograph of Jerndal et al (1978) rs more information regarding the role of dominant goniodysgenesis (Fig 3)

hree generations are afflicted and the glaucomas were discovered between 45 l 55 years of age except for case III 1 – a myopic male whose glaucoma was gnosed at 32 years All three glaucomatous members examined by us had typical iodysgenesis

I 4 in addition had exfoliation phenomena and III 1 the classic clinical picture pigmentary glaucoma

hus this family at first sight appeared to have a dominant simple glaucoma but closer examination demonstrated dominant goniodysgenesis combined with foliation and pigmentary syndromes

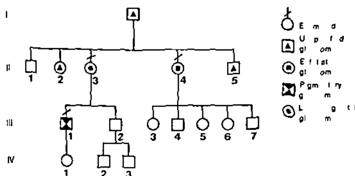


Fig 3

ndal et al (1978) Pedigree with dominant glaucoma presenting late congenital glaucoma exfoliation glaucoma and pigmentary glaucoma all with typical goniodysgenesis

Discussion

A summary of pertinent literature and the presented pedigrees [illegible] open angle glaucoma may exist as a dominant disorder and that [illegible] occur in early infancy and in fact lacks an upper limit The primary [illegible] evidently a maldevelopment of the irido-corneal angle [illegible] discussed and highlighted with scanning electron micro[illegible] by Jerndal [illegible] (1978) If the dysgenetic changes are pronounced infantile or [illegible] may ensue With less advanced goniodysgenesis additional factors [illegible] a gradually impaired outflow and eventually precipitate glaucoma [illegible] several such factors [illegible] the pigmentary liberation syndrome [illegible] syndrome steroid provocation and hyphaema

Many ophthalmologists have doubted that congenital g[illegible] a delayed onset of elevated IOP and glaucoma late in life It is however [illegible] clinical experience that congenital anomalies can cause late functional [illegible] e g stenosis of the renal artery and arterial hypertension stenosis of [illegible] of Sylvii and hydrocephalus congenital cystic kidneys and renal fa[illegible]

Another interesting finding by Jerndal & Munkby (19[illegible]) is that [illegible] high tensional response on provocation with topical steroids [illegible] inherited and appears correlated to the presence of goniodysgenesis.

References

Andersson J R (1939) *Hydrophthalmia or congenital glaucoma* [illegible] Press

Armaly M F (1961) The heritable nature of dexamethasone induced [illegible] *Arch Ophthal (Chicago)* 75 32

Becker B (196[illegible]) Intraocular pressure response to topical [illegible] 198

Berg F (1932) Erbliches jugendliches Glaukom *Acta ophthal (Kbh)* [illegible]

François J (1961) *Heredity in ophthalmology* [illegible] Mosby St Louis

Gillespie F D (1963) Congenital glaucoma juvenile glaucoma [illegible] one family *Virginia Med Mth* 90 5 6

Jerndal T (1968) Familial congenital glaucoma with dominant [illegible] (Kbh) 46 [illegible]

Jerndal T (19[illegible]) Goniodysgenesis and hereditary juvenile glaucoma [illegible] *Suppl* 10[illegible]

Jerndal T & Munkby M (1978) Corticosteroid response in dominant [illegible] *Acta ophthal (Kbh)* 56 3[illegible]

Jerndal T Hansson H A & Bill A (19[illegible]) [illegible] glaucoma Scriptor Copenhagen

denburg P J Franceschetti A & Klein D (1961) *Genetics and Ophthalmol gy* Vol
) 518 Blackwell Oxford
4] C F (1966) *The pathogenesis of c ngenital glauco a* van Gorcum Assen

r s add ess

anders Lund Ogonkliniken
renska Sjukhuset 413 45 Goteborg Sweden

ACTA OPHTHALMOLOGICA VOL. 57 1979
XXIV MEETING OF NORDIC OPHTHALMOLOGISTS
Oslo Norway 13–16 June 1979

Department of Ophthalmology II Head F Enoksson
University of Göteborg Göteborg Sweden

CONTRAST SENSITIVITY IN MACULAR DISEASE USING A SMALL-FIELD AND A LARGE FIELD TV SYSTEM

BY

JOHAN SJÖSTRAND

The spatial contrast threshold for a sinusoidal grating of varying (or ...) frequency generated with a small field or a large-field TV display was ... in normal subjects and in patients with macular disorders
Impairment of contrast sensitivity (reciprocal of contrast) was observed ... different maculopathies investigated Attenuation of the high and ... frequency ranges was an early finding in macular disease whereas ... including the low frequency range was observed in more advanced ... pathies Comparison of results obtained using the small (1.4°) or large (6–24°) TV system demonstrated a field-dependence of the contrast sensitivity attenuation in localized macular disorders In more widespread lesions ... the posterior pole a contrast attenuation over the whole frequency range ... found also with the largest (24°) stimulation field used
The study of the contrast sensitivity function supplements the ... acuity measurements in quantifying the visual loss for objects larger than the resolution limit I conclude that contrast threshold measurements are not only useful for describing visual loss but also for tracking progression of ... The findings provide some additional insight into the visual ... life of patients with a macular disorder However the definite role of ... sensitivity measurements for diagnosing different visual disorders ... lacking

Key words contrast sensitivity – maculopathy – spatial frequency ... acuity

The commonly used acuity tests give information about the ... fine targets at high contrast The ability to see larger targets and ... contrast is of equal importance and is usually not tested in clinical ...

-<

clinical practice we know however that patients sometimes complain of oration of low-contrast vision even though their visual acuity is within normal : A more general description of the visual system is obtained by studying the ast sensitivity function i e the reciprocal of threshold contrast for a range of oidal gratings that vary in spatial frequency (for review see Campbell 1974 ar 1974) By the contrast sensitivity it is possible to study visual functions not l in the acuity test.

e aim of the study has been to investigate the contrast sensitivity function in us maculopathies and the interest has been focused on the following question ntrast sensitivity measurement a useful additional clinical test in the diagnosis nent and follow up of macular disorders?

Material and Methods

nts

n normal controls (age 19–63 years) and 22 patients (age 19–78 years) with lar disease of different types took part in this study

field TV system

al sinusoidal gratings were generated on a television display as pre ously described by and & Frisen (1977) The television monitor was masked to subtend 1 4 × 1 4 degrees of at the eye when viewed at a distance of 5 m A white illuminated mask which nded approximately 2×2 degrees surrounded the TV screen Spatial frequency ber of cycles per degree of visual angle) was varied between 1 5 and 38 cycles degree m contrast (defined as ($L_{max} + L_{mi}$) where L_{max} and L_{min} are the maximum and num luminances of the sinusoidal pattern) was varied in 2 dB steps in the 0 65–0 000 – The space average luminance of the gratings was 135 cd m²

e contrast sensitivity (reciprocal of contrast threshold) was determined monocularly with nal correction and natural pupil Seventeen frequencies were explored twice in a random ner nd the contrast threshold determined by raising the contrast from a sub-threshold t a selected spatial frequency until a pattern was just visible by the patient

e-field TV system

90 TV monitor used in the large field system was masked to subtend 6 × 6 degree of e at the eye when viewed at a distance of 2 5 m The TV monitor was surrounded by a / screen and by illumination of two daylight tubes a uniform background luminance of d m² was obtained The space average luminance of the grating was 83 cd m The spatial uency was varied between 0 5 and 30 cycles degree The pattern contrast of the soid l grating was varied in 2 dB steps in the 0 6–0 001 range

y changing the distance to the monitor the size of the TV screen was increased to 24–24 rees of angle at a distance of 0 6 m The size of the retinal images using the field size of 6 and 24 respectively is illustrated in Fig 1 The test distance was controlled by using a n support and refraction was optimally corrected for the distances used The test cedure was as described above for the small field system

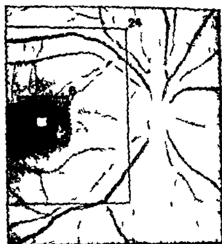


Fig 1

A fundus picture of a normal control with a schematic drawing of the retinal images of TV screen which subtended 1 4 (□) 6 and 24 degrees of arc.

Results

Normal subjects

Small field TV system The contrast sensitivity curves (CSC) i.e. the contrast sensitivity plotted as a function of the spatial frequency of the sinusoidal grating had bell shaped form when tested for subjects with normal vision no previous disease and ophthalmoscopically healthy eyes (Sjostrand & Frisen 1977) in agreement with previous studies (Schade 1956 Campbell & Robinson 1968). The curves showed a high and low frequency attenuation with a peak sensitivity between 8 and 15 cycles/degree (Figs 2–4) Our CSC curves in normal controls using the small field TV system (1 4°) differ from those obtained using the large field TV system (6–24) and from those previously reported in that our subjects peak sensitivity occurred at a higher spatial frequency

Large field TV system The contrast sensitivity curves of 10 normal controls obtained using the large field TV system had a much broader peak with a peak region varying from 1 to 8 1–10 and 2–12 cycles/degree for a field size of 6 1° and respectively (Figs 7 and 8) A comparison of the peak frequency obtained for normal controls measured on both the small field (1 4°) and the large field TV system masked down to 1 4 (see legend Fig 7) showed a mean difference of cycles/degree for the two systems used (cf Figs 2 and 7) The difference in peak frequency therefore presumably reflects the mask and the surroundings and the luminances (cf Estevez & Cavonius 1976)

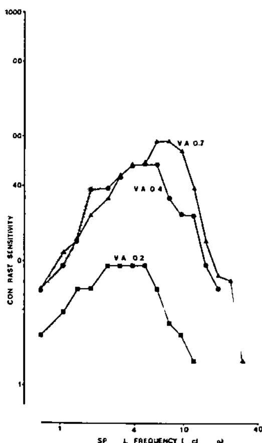


Fig 2

SC (1 4°) in *normal controls* (dotted area indicates range for ten normal subjects aged 9 and 61 years) and three representative cases of different stages of *senile macular egeneration* (solid lines) Case I A (▲ female 65 years-old) had moderate macular changes and V A 0 7 in case K L (● male 46-years-old V A 0 4) changes were more advanced and in case L.L (■ male 65 years-old V A 0 2) the changes were extensive Data of normal controls from Sjostrand & Frisen (1977)

lar d sorders – small field TV system

e CSC was measured in different types of macular disease senile macular eneration central serous retinopathy fundus flavimaculatus vitelliform macular eneration and retinal detachment including the macula The macula is mor ologically and functionally non uniform and since macular disorders may involve foveal perifoveal and perimacular region or the receptor or neuronal cells

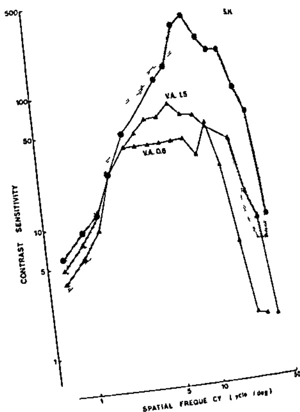


Fig 3

CSC (1 4) in *central serous retinopathy* Case S H (male 49 years old) with involvement of the right eye (▲ lower curve was obtained during the acute phase V A 0 6 and the upper curve following recovery V A 1 5) The CSC of the contralateral left eye (V A. 1.5) is indicated by filled circles (●) Dotted area shows the range of seven normal controls aged 31 to 63

differently variation in effects on the contrast sensitivity function may be anticipated and is in fact demonstrable in this study

Senile macular degeneration In senile macular degeneration pathological changes occur within the macular area (Sarks 1976) giving rise to scotomata of various in central vision

The CSC was impaired in senile macular degeneration and the decrease notable at high and intermediate frequencies in cases with reductions in visual acuity to 0 5–0 7 (Fig 2 case I A V A 0 7) In advanced cases of degeneration abnormality of CSC across the whole spatial frequency range observed as illustrated by case L L (V A 0 2 in Fig 9)

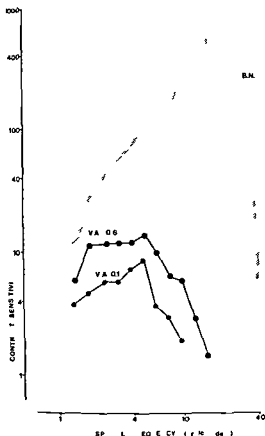


Fig 4

B N female 24-years-old CSC (1 4°) of the left eye (●) of this patient with *fundus maculatus* with macular involvement (Stargardt s disease) The upper curve was obtained 76 (V A 0 6) and the lower curve after a period of progressive deterioration of central in 2½ years later (V A 0 1) The dotted area represents the range of five normal subjects aged 19 to 31 years (from Frisen & Sjostrand 19 8)

n 2 patients with macular oedema at the time of the initial test, a subjective provement and also an improved CSC was demonstrated at a second determina n 3–6 months following the initial test

A rough correlation was seen between the contrast sensitivity level between 5 and cycles/degree and the visual acuity in patients selected to be in a stationary phase disease with no obvious retinal oedema (results not shown)

ntral serous retinopathy In this macular disorder macular oedema develops due to akage through the pigment epithelium In most cases tested a detachment of the

pigment epithelium in the macular region (Gass 1967) was observed. During the acute phase the patients complained about impaired discrimination of fine details, metamorphopsies and micropsia (Frisen & Frisen 1979)

CSC was followed during the course of the disease and during the acute phase contrast sensitivity was decreased for high and intermediate frequencies. Generally the CSC improved parallel to the increase of visual acuity. Fig 3 demonstrates changes in CSC in one case during acute and recovery phase. It is notable that during resolution when the visual acuity had improved to 1.5 the patient complained about slight blurring of the vision and the CSC was still markedly abnormal in the high and mid frequency region (Fig 3)

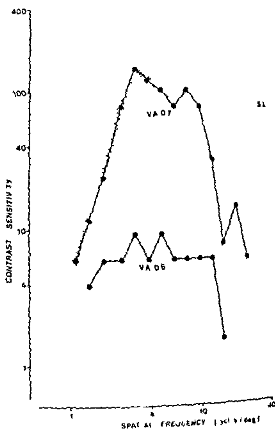


Fig 5

Case S L male 45 years old CSC (1 4) of the left eye (●) of this patient with *macular degeneration* with slowly progressive deficit in central vision. *The upper curve* obtained in 1976 (V A 0 7) and the lower curve (V A 0 6) 2½ years later. *The dotted area shows* the range of seven normal controls aged 37 to 63 years

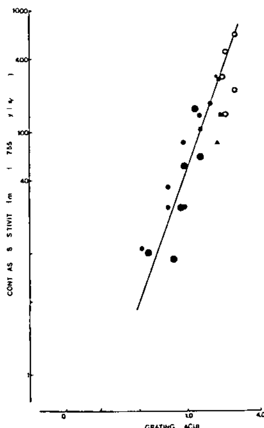


Fig 6

The relation between the mean contrast sensitivity in the mid frequency range (.55 12 cycles/deg) obtained with the small field TV system (1 4) and grating acuity Grating acuity was measured monocularly by means of sinusoidal gratings displayed on the TV screen at a distance of 7 9 m (for details see Andersson & Sjöstrand 1979) Fifteen cases with healed retinal detachment involving the macula were examined ★ normal controls 19 to 42 years-old ○ normal controls 43 to 70-years-old ● macular detachments aged 19 to 41 years • macular detachments aged 42 to 70 years ▲ fellow eyes for patients aged 19 to 41 years ■ fellow eyes for patients aged 42 to 70 years

Miscellaneous maculopathies In this group various progressive maculopathies such as fundus flavimaculatus (Stargardts disease) and vitelliform macular degeneration were investigated During the time period of this study (3 years) the visual acuity dropped in several of the patients studied Fig 4 demonstrates the CSC-curve of a 4-year-old female with a fundus flavimaculatus involving the macular region

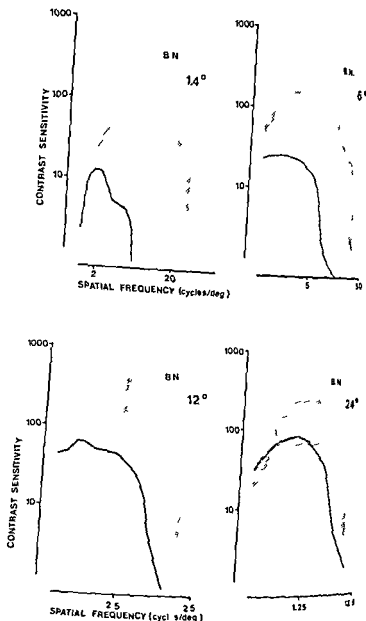


Fig 7

Case B N with *fundus flavimaculatus* (the same patient as described in Fig 4) CSC of the eye (solid line V A 0 1) of this patient using the *large field* TV system (6–24°). The dotted area shows the range for 5 (6) and 6 (12 and 24) normal controls. To investigate the CSC at an angle of 1 4 at the eye (the same angle as that used with the small field TV system) the TV system ordinarily used for large field the screen was masked to 1 4 with a cardboard mask with the same background luminance as the surroundings. Dotted area in the 1 4 field indicates the range for six normal controls a) 1 4 and 6 b) 1 and 24

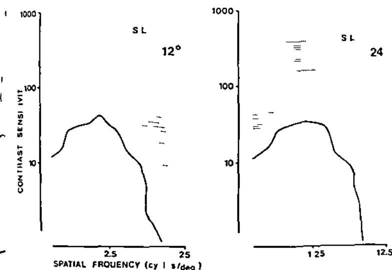


Fig 8

…e S L with *itell form macular degene ation* (the same patient as described in Fig 5) CSC of left eye (solid line V A 0 6) with the *large field* TV system (1° and 24) The CSC for 6 s even more severely depressed (results not shown) Dotted area shows the range for six normal subjects

…ready 3 years ago when the visual acuity was 0 6 the CSC curve was impaired …d, when studied half a year ago both the visual acuity (0 1) and the CSC had …opped further (Fig 4)

Another patient with vitelliform degeneration of the macula studied during the …ne time period is shown as comparison During the time period of study visual …uity only decreased from 0 7 to 0 6 whereas the CSC depression was markedly …gmented over the whole frequency range (Fig 5) At the same time the patient …perienced increasingly severe metamorphopsies and the macular status deterio-…ted as visualized by the ophthalmoscope

…tinal detachment including the macula We have investigated a number of patients …th rhegmatogenous retinal detachment including the macula and followed the …tinal function with various tests postoperatively (Andersson & Sjostrand 1979) It …known that age and refraction of the patient as well as duration of macular …etachment preoperatively are of great importance for the functional outcome …undry & Davies 1974 Kreissig 1977)

Following a successful operation of macular detachment an initial rapid increase … visual acuity is found followed by a second phase of prolonged visual improve…nent extending over more than a year (Kreissig 1977) The spatial processing in the

reattached macula was followed by measuring the contrast sensitivity fu[illegible] and grating acuity during the recovery phase

In a group of patients examined about a year after the operation [illegible] CSC could be observed in the two age groups studied (Andersson & Sjö[illegible] 1979) The high and intermediate frequency ranges were affected predo[illegible] Within the low frequency range the contrast sensitivity essentially was within the normal range The reduction of the contrast sensitivity in the mid frequency r[illegible] was roughly correlated to the visual acuity and grating acuity in these patients [illegible]

Macular disorders – large field TV system

To obtain a better insight into the field related visual problems of patients [illegible] visual disorders a few of the patients measured with the small field TV system were selected for further study using a large field TV system with a visual angle of [illegible] 24 subtended at the eye

The functional area for summation to threshold for sinusoidal grat[illegible] function of both the length of the bars and the number of bars of the gr[illegible] (Howell & Hess 1979) By using a field of 24 the length of the bars and the n[illegible] of cycles are above the critical limit at 0 5 cycles/degree giving rise to a valid contrast sensitivity function at this spatial frequency and above it With the smaller field[illegible] used an artefactual depression of the low spatial frequency end of the co[illegible] sensitivity function is introduced (Estevez & Cavonius 1976 Howell & Hess 19[illegible] However in more localized macular lesions a small field size is important in order to assess contrast sensitivity changes in fovea near regions of the macula

An illustrative case is the 24 year-old woman (B N cf Fig 4) with fun[illegible] flavimaculatus with a severe macular involvement giving her a visual acuity of 0 1 [illegible] the time of test with the large field TV system As demonstrated by Fig [illegible] a marked depression of the whole CSC curve is found for a field size of 1 4 and 6° using the TV system At an angle of 24° subtended at the eye the CSC curve is almost normalized at lower spatial frequencies demonstrating the striking field dependence that is found in some of the maculopathies studied In the case with [illegible] macular degeneration (case S L) however the CSC depression at lower [illegible] frequencies was observed also with the largest screen size used (Fig 8) indicating more extended lesions in the posterior pole of the eye

Discussion

During the last decade psychophysical and evoked response methods have been used to measure the capacity of the human visual system to observe con[illegible] patterns (Campbell & Maffei 1970 Campbell 1974 Sekular 1974) [illegible]

odic gratings of variable contrast have been used as targets to study the ability to eive low-contrast patterns under various experimental and clinical conditions as been suggested that there may be in the visual system channels tuned to ow ranges of spatial frequencies i e neurons exist in the visual system which selectively sensitive to certain sizes of visual targets (Blakemore & Campbell 9 Campbell & Maffei 1970 Campbell 1974) Our knowledge of the contrast on in disease however is more limited (for discussion see Arden 1978)

he results reported here have demonstrated that measurement of contrast is only useful for describing visual loss for objects larger than the resolution limit also of value for tracking progression or recovery in agreement with a liminary study (Sjostrand & Frisen 1977) The slope of the line in Fig 6 relating trast sensitivity to grating acuity in postoperative cases of macular detachment icates that the contrast sensitivity measurement is as sensitive as grating or any er acuity

n early stages of maculopathies mainly the contrast sensitivity of the high and ldle frequency range was attenuated using the *small field TV system* In advanced s of maculopathies the pattern of contrast sensitivity depression was similar to t in acute cases of optic nerve disease (Frisen & Sjostrand 1978) and anisome pic amblyopia (Sjostrand 1979) with impairment over a wide range of spatial quencies

n spite of the disadvantages of using the small TV screen this system assessed contrast sensitivity function of the central spatial vision without interference of more peripheral vision It has been shown that contrast sensitivity along a tical line through the fixation point falls off steadily from a maximum at the tre (Robson & Graham 1979) However this decline with eccentricity is most rked at high spatial frequencies and is small at lower spatial frequencies The es with an impairment over the whole spatial frequency range using the all field system presumably has an involvement of the central area subtending selective impairment of high spatial frequencies indicate that a peripheral part the 1 4 stimulation field is spared

Using the large field TV system with a field size ranging from 6 to 24 it was ssible to study the contrast sensitivity when retinal summation was allowed over a ger field A strong field-dependence of the CSC-deficit was found in many of the aculopathies restricted to the central part of macula In such cases the contrast nsitivity function at lower spatial frequencies was almost normal with grating ld subtending an angle of 24 at the eye (Fig 7) In cases with extensive lesions in e posterior pole a subnormal CSC over the whole frequency range was still seen th a large stimulation field Similar findings have been found in the acute phase f optic neuritis *The CSC impairment over the whole frequency range appears to* e compatible with a disturbance of a majority of the afferent visual channels that

subserve central vision The differential field-dependence provides some [illegible] into daily visual problems of patients with macular disorders Hess et al. [illegible] investigated a case with a uniocular central 6 scotoma and they conclude that the macular region contributes significantly to the visibility of objects as large as [illegible] A marked impairment of the low spatial frequency end of the contrast sensitivity curve however indicates involvement of a much larger area (cf Robson & [illegible] 1979) The present study emphasizes that it is important to test CSC at different discrete regions over the visual field to assess regional differences

Previous studies of contrast sensitivity with stimuli of sinusoidal gratings displayed on a TV or oscilloscope screen have described abnormalities in contrast detection in patients with myopia (Fiorentini & Maffei 1976) amblyopia (Freeman & Thibos 1975 Levi & Harwerth 1977 Hess & Howell 1978) and suprageniculate lesions of the visual pathways (Bodis Wollner 1972 1974 1976) In a recent study using a book of printed gratings Arden & Jacobson (1978) have demonstrated subnormal contrast sensitivity in glaucomatous patients

The majority of these studies give no evidence for a selective vulnerability [illegible] various spatial frequency ranges The pattern of CSC attenuation is generally similar for a number of clinical disorders with mainly high frequency loss in disease such as corneal oedema (Hess & Garner 1977) strabismic amblyopia (Sjöstrand 1969) retinitis pigmentosa (Wolkstein et al 1977) and early maculopathy [illegible] study) and more general depression of CSC in optic nerve disease (Frisén & Sjöstrand 1978) anisometropia (Sjöstrand 1979) and advanced maculopathy [illegible] study)

In conclusion our study has shown that measurement of spatial contrast sensitivity in visual disorders supplements traditional visual acuity measurements and is useful for tracking progression or recovery of macular disease Based on the present findings a less time-consuming test might be constructed using [illegible] selected spatial frequencies at high intermediate and low frequencies. However the definite role of CSC for diagnosing or distinguishing different clinical disorders [illegible] is lacking The possibilities to use contrast measurements other than at threshold should therefore be explored In our laboratory we are currently investigating the effect of disease on the supraliminal contrast level.

Acknowledgments

Supported by the Swedish Medical Research Council (grant [illegible]) [illegible] Reumatism and Carmen and Bertil Regner s Foundation

I am grateful to Dr L Frisén for valuable advice and constructive discussion and for the loan of his television equipment

References

ersson C. & Sjostrand J (19~9) The function of the macula following retinal detachment olving the macula. *Acta ophthal (Kbh) 57* (in press)

n G B (19~8) The importance of measuring contrast sensitivity in cases of visual sturbance *Brit J Ophthal 62* 198–?09

n C B & Jacobson J J (19/8) A simple grating test for contrast sensitivity Preliminary sults indicate value in screening for glaucoma *Inve t Ophthal V s Sci 17* 23–32

emore C & Campbell F W (1969) On the existence of neurons in the human visual stem selectively sensitive to the orientation and size of retinal images *J Phy i l (Lond.)* 13 ?3 –?60

s Wollner I (19/4) On dissociated visual functions *Mou t S i n Med 41* 38–45

s-Wollner I (19 6) Vulnerability of spatial frequency channels in cerebral lesions *Natu e ond) ?61* 309–311

pbell F W (1974) The transmission of spatial information through the visual system *The eurosciences Thi d Study Program*, pp 95–103 The MIT Press Cambridge Mass and ondon

apbell F W & Maffei L (19~0) Electrophysiological evidence for the existence of ientation and size detectors in the human visual system *J Physiol (Lond.) 207* 635–652

apbell F W & Robson J G (1968) Application of Fourier analysis to the visibility of ratings *J Physol (Lond) 197* 551–556

nez O & Cavonius C R (1976) Low frequency attenuation in the detectation of gratings orting out the artefacts *V is Res 16* 497–500

renum A & Maffei L (1976) Spatial contrast sensitivity of myopic subjects *V is Res 16* 3/–438

eman R D & Thibos L N (1975) Contrast sensitivity in humans with abnormal visual xperience *J Phy i L (Lond) 247* 687–710

sen L & Sjostrand J (1978) Contrast sensitivity in optic neuritis *Docum. Ophthal P oc Ser* 165–1/4 Dr W Junk b v Publishers the Hague

sen L & Frisen M (1979) Micropsia and visual acuity in macular edema A study of the euroretinal basis of visual acuity *Albrecht v Graefe Arch kl n exp Ophthal 210* 69–77

s J D M (196/) Pathogenesis of disciform detachment of the neuroepithelium II diopathic central serous choroidopathy *Amer J Ophthal 63* 58~–615

ndry M F & Davies E W G (1974) Recovery of visual acuity after retinal detachment surgery *Amer J Ophthal /~* 310–314

ss R F & Howell E R (1977) The threshold contrast sensitivity function in strabismic amblyopia evidence for a two type classification *V is Res 17* 1049–1056

ss R F Jacobs R J & Vingrys A (1978) Central versus peripheral vision Evaluation of the residual function resulting from a uniocular macular scotoma *Amer J Optom 55* 610–614

reussig I (19/~) Prognosis of return of macular function after retinal reattachment *Mod P obl Ophhal 18* 415–429

ri D M & Harewerth R S (1977) Spatio temporal interactions in anisometropic and strabismic amblyopia. *Invest. Ophthal. V is Sci 16* 90–95

obrn J G & Graham N (1979) Probability summation and regional variation in contrast sensitivity across the visual field *V is Res* (in press)

arks S H (19 6) Ageing and degeneration in the macular region A clinicopathological study *Brit J Ophthal 60* 324–341

Schade Sr O H (1956) Optical and photoelectric analog of the eye J Opt Soc Amer 46 721–739

Sekular R (1974) Spatial vision *Ann rev Psychol 25* 195–232

Sjostrand J & Frisen L (1977) Contrast sensitivity in macular disease. A preliminary report *Acta ophthal (Kbh) 55* 507–514

Sjostrand J (1978) Contrast sensitivity in amblyopia. A preliminary report 2 135–137

Wolkstein M Atkin A & Bodis Wollner I (1977) Grating acuity in two cases of tapetoretinal degeneration *Docum Ophthal Proc Ser* pp 41 46, Dr W Junk Publishers the Hague

Author s address

Dr J Sjostrand Department of Ophthalmology
Sahlgren s Hospital S-41345 Goteborg Sweden

ACTA OPHTHALMOLOGICA VOL 57 1979

The Eye Depart nent Rig ho pite l t Ct pe shagen[1] a l
The Eye Path l gy In titute L u er ity f Ct pen hagen C pe hage i[2]

MORPHOLOGICAL HISTOCHEMICAL AND X RAY MICROANALYTICAL EXAMINATION OF DEPOSITS ON SOFT CONTACT LENSES IN EXTENDED WEARING

BY

V DREYER O A JENSEN[2] and J U PRAUSE[2]

The deposits on 99 contact lenses of various water content from 90 wearers were analysed by the methods given in the title Six lenses were used as bandage lenses the remaining for optical correction The age of the patients varied from 7 to 56 years two-thirds being under 45 years
Wearing time had been from 1 week to 1 year with an a erage of 13 weeks
The results obtained by the methods applied showed that calcium was present in just over two-thirds of the cases (90/29) other elements being infrequent
Mucopolysaccharides were found in just under two-thirds (18/99) Chlorine was present in one fifth of the cases No s gnificant amounts of lipid were detected Fungi were found in three cases (3 99) Bacteria were also found in these cases but never without fungi
Evaluation of the methods applied sho ed that the methods of choice were macroscopical examination and scanning electron micr scopy in c mbination with X ray microanalysis in a few cases combined with histo hemi try None of the methods applied is sufficient for pr tein analysis

Key u rd soft contact lenses – extended wearing – deposits – histc chemistry – electron microscopy – X ray microanalysis

posits on soft contact lenses (CL) are an ever increasing source of unsuccessful aring Knowledge of the nature of deposits is a mandatory prerequisite for the commendation of effective cleansing methods with the object of prolonging the e of lenses

---

ead in a modified and abbreviated form at the 8th European Congress of Med cal ontactology Strasbourg France 6–8 Oct 1978

eceived October 19 19 8

Table I

Previous examination of deposits

Year	Author(s)	Number	Type of lens
1972	Matas et al	3	Various
1973	Tragakis et al	55	Hema, PVP
1973	Tripathi & Ruben	1	Bionite
1975	Doughman et al	19	B-L soflens Bocca *
1975	Lowther	5	
1975	Ruben et al	25	Various**
1976	Hilbert et al	5	B-L soflens
1977	Cochet	969	Various **
1977	Kanai et al	18	[illegible]
1977	Klintworth et al	9	Soflcon
1977	Wedler	10	B-L soflens
1978	Allen et al	6	Sauflon 85

* Griffin Kontur Bausch & Lomb
** Sauflon Bionite Poly Hema, PMMA
*** Unspecified
(n) Indicates n lenses examined by the method

Thorough examination of the deposits applying various physical chemical and morphological methods has been carried out on a somewhat limited number of lenses (Table I)

The present study was planned to include a combination of light microscopy and histochemistry (LM) transmission electron microscopy (TEM) scanning electron microscopy (SEM) and X ray microanalysis (X ray) of deposits on contact lenses of various water content worn for extended periods to ascertain the composition of these deposits and to evaluate which methods were appropriate for routine use.

Material and Methods

A total of 29 hydrophilic lenses from 20 patients was examined Table II lists the ages of the patients with a range from 7 to 86 years two-thirds being under 45 years of age Most of the patients wore CL because of myopia only five because of corneal or conjunctival disorders The continuous wearing period had varied from 1 week to 1 year with an average of 13 weeks All lenses had been discontinued because of troublesome and unremovable deposits

Table I (cont)

ft contact lenses

	Methods of examination									Results
r	Bioch	Cult	LM Histo-chem	SEM	TEM	X ray micr	X ray dif	Atom Abs	Spect phot	
				+						Bact
		+	+							Bact
					+					Debris Bact?
	+(6)		+(3)	+(8)	+(2)					Lip
			+				+			Pr MPS Ca
	+		+	+	+	+	+(2)	+	+	$Ca_3(PO_4)_2$ Si S Cl
			+							Pr MPS Ca
										Pr Fu
					+	+				Ca Fu
	+		+			+				Ca Pr
	+									Pr MPS Lip
			+(1)					+(?)		MPS Lip Ca

eviations

light microscopy SEM scanning electron microscopy
M transmission electron microscopy Pr protein MPS mucopolysaccharides
lipids Fu fungi

-thirds of the lenses were of the SCANLENS 4th type a PVP PMMA (polyvinylpyrol- one polymethyl methacrylate) with a water content of 76%

The lenses were stored at 4 C until use Upon examination each lens was photographed in and in sectors in a photomacroscope (Wild M 400) using light field and dark field mination All lenses were processed according to routine paraffin technique The last half the series was sectioned and appropriate parts were also processed for LM as well as for M and TEM

Routinely lenses were fixed in 4% phosphate buffered neutral formaldehyde for 24 h at dehydrated in ethanol and paraffin embedded In some cases half a lens was cut in a ostat (Am Opt Corp) and stained with Luxol fast blue (Luxol) Sudan black B (black B) d oil red O (red O) Deparaffinized sections were stained with haematoxylin-eosin (HE) ematoxylin phloxine safranin (HPS) periodic acid Schiff (PAS) Alcian blue with 0 9 M and M $MgCl_2$ (AB) alizarin red S (Aliz) the von Kossa method (Kossa) murexid staining a m ufmann & Adams (Mur) silver nitrate rubeanic acid a m Yasue (Rub) the oxidized nnin azo method (OTA) Grocott s modification of Gomori s methenamine silver nitrate chnique (Grocott) Gridley s method for fungi (Gridley) and Brown Brenn s bacterial stain B)

For SEM critical point dried (acetone CO_2) air dried or formalin vapour exposed ecimens were used Specimens without or having a sputter coating of Au/Pa of 300–400 Å

Table II
Age of patients diagnosis wearing time and type of lens

Patient No	Lab	Sex	Age	Diagnosis	Wearing time (weeks)	Type of lens
1	415/77	F	61	Myopia		Plano-T
2	457/77	M	70	Pemphigus –	2	Plano-T
2	458/77	–	–	Conj	–	
3	485/77a b	F	40	Myopia	4	Scanlens
4	486/77a b	F	28	Myopia	4	Scanlens *
5	487/77a b	M	20	Myopia	3	Scanlens *
6	488/77	M	29	Myopia	9	Scanlens *
7	495/77	M	13	Keratitis –	1	Scanlens *
7	517/77	–	–	neuroparalytica	1	
8	706/77	F	86	Keratitis bullosa		Plano-T
9	778/77	M	19	Aphakia	24	Weicon
10	1198/77	M	27	Myopia	59	Weicon
11	1238/77	M	7	Anisometropia		Scanlens *
12	1244/77	M	76	Keratitis bullosa	90	Weicon
13	99/78	M	71	Aphakia	15	Scanlens *
14	116/78a b	M	26	Myopia	9	Scanlens *
15	163/78a b c d	F	20	Myopia	5	Scanlens *
16	230/78					Scanlens *
17	251/78	F	21	Myopia	6	Scanlens *
18	266/78	M	30	Myopia	98	Scanlens *
19	307/78	F	42	Myopia	7	Scanlens *
20	351/78	M	41	Myopia	21	Scanlens *

a b Means a pair of lenses
a b c d Mean two pair of lenses worn for the same period
In cases 2 and 7 the two lenses indicated in the table are from the same eye No clinical data of patient No 16 were available

thickness were examined The SEM specimens were used for elemental analysis A total of 4 lenses was scanned and microanalysed For TEM 1 mm² parts with deposits were cut out of the lens fixed in 4% cacodylate buffered glutaraldehyde at room temperature for h post fixed in 2% OsO_4 for 2 h dehydrated in graded acetone and embedded in a mixture of epon Survey sections of 1 µm and ultrafine sections were cut with glass knives or by diamond knife in a Reichert ultramicrotome OM U3 the survey sections being stained with toluidine blue and the fine sections with 1% uranyl acetate for 45 min and 1% lead citrate for 3 min Five lenses were used for TEM

SEM was performed in the Jeol JSM 35 at 15 10 and 7 kV and TEM in either the Zeiss EM 9 S-2 at 60 kV or the JEOL 100 C at 60–80 kV Elemental analysis was carried out in the JEOL analytical Temscan 100C/100CX at 40 kV

A survey of the technical procedures is given in Table III

Table III
Technical procedures

Macroscopical examination
a Examination in dissecting microscope
b Macrophotohraphy
1 light field
2 dark field
Histopath.-histochem. examination
a Paraffin sections
b Frozen sections
c Staining
HE HPS PAS AB Aliz Kossa Mur Rub OTA
Crocott Gridley B B Luxol black B red O
SEM
a Air dried — coated / uncoated
b Formalin vapour exposed — coated / uncoated
c Critical point dried — coated / uncoated
X ray — microanalysis
Performed on SEM specimens
TEM
Glutaraldehyde – araldit/epon

Results

able IV is a summary of the methods applied in the individual cases with a survey the composition of the deposits as interpreted from the study Macroscopical xamination (Fig 1) and light microscopical histochemistry were performed in all ases TEM in five and SEM and X ray microanalysis in 14 cases Initially when LM as exclusively used we interpreted a positive AB alone or together with a positive AS as manifestation of MPS but not a positive PAS alone To accept the presence f calcium using LM alone at least two of four methods for Ca (Aliz Kossa Mur ub) should be positive Sulphate—containing MPS were considered present when spectrum containing a peak for S was found together with positive histochemical tainings (Fig 2) Although the histochemical stainings were negative MPS might e the source of the sulphur in the event of an S peak However we avoided such a ecording Prominent Cl peaks were found in nine cases together with traces of

Table IV
Survey of methods and results

Patient No	Lens No	LM	TEM No	SEM No	X ray	Type of deposits
1	1	+				
2	2	+				Ca, MPS
2	3	+				Ca, MPS
3	4	+				Ca, MPS
3	5	+				Ca, MPS
4	6	+				Ca, MPS
4	7	+				Ca, MPS
5	8	+				Ca, MPS
5	9	+				Ca, P, MPS
6	10	+				Ca, P, MPS
7	11	+				Ca, MPS
7	12	+				Ca, Pr
8	13	+				Ca
9	14	+				Ca
10	15	+				MPS
11	16	+		242	+	MPS, Fa, [illegible]
12	17	+	251	251	+	MPS, Fa, [illegible]
13	18	+	253			Ca, MPS
14	19	+		263	+	Pr
14	20	+	269	264	+	Ca, Mg, Cl, [illegible]
15	21	+	269	269	+	
15	22	+		271A	+	Na, Cl
15	23	+		271B	+	Ca, Na, Cl
15	24	+		275	+	Na, Cl, Fe, [illegible]
16	25	+	276	276	+	Na, Cl, S
17	26	+		280	+	Ca, Na, K, [illegible]
18	27	+	281	281	+	Ca
19	28	+		286	+	Ca, Na, Cl, [illegible]
20	29	+		299	+	Ca, Cl, [illegible]
		+		295	+	Ca, Cl, S, [illegible]
Total examinations		29	5	14	14	

Abbreviations see Table I

other elements and in six cases together with sodium In the latter cases the salt may be sodium chloride from tear fluid storage fluid or lens material. Stains for protein were only positive in two cases One of these cases was also analysed by X ray microanalysis without definite peaks This was expected, since light elements such as carbon oxygen and nitrogen the main elements of proteins cannot be significantly detected by electron beam X ray microanalysis The same applies to lipids Lipids in significant amounts were not detected in the frozen sections.

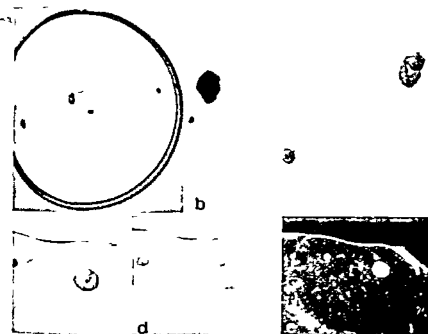


Fig 1

roscopical view of smooth globular deposits in light field and dark field.
hole lens (Lab No 116b/78 × 4)
etail of a. (× 12)
eposit in transmitted light (Lab No 1244 (× 30))
ame in transmitted tangential light (× 30)
ame in dark field illumination (× 20)

ollowing the above principles the analyses showed that calcium was present in possibly 21 out of 29 lenses (Fig 3) although in varying amounts (Table IV) is was to be expected as the composition of the deposits varied on the same lens s noticeable that calcium and fungi do not particularly go together (Table IV) wever bacteria were present in the three cases of fungal contamination as if the ro-organisms were in symbiosis (Fig 4) It is impossible to judge from morph gy light microscopical or electron microscopical methods or by staining thods the exact kind of fungi but probably aspergillus was involved in at least e case The bacteria had the shape of small rods

MPS were found in 18 out of 29 lenses However three of these cases were less nificant because of insufficient material for analysis Only biochemical analysis ntenfy any linkage with protein

Light microscopically and in SEM the most common type of deposit was round d smooth (Figs 1 and 3) More rarely it had a crystalline appearance (Fig 2)

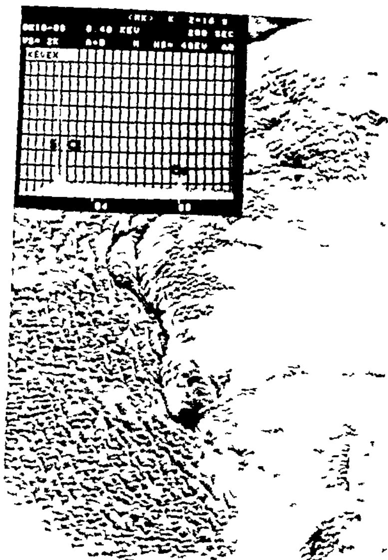


Fig 2

Crystal like deposit containing MPS The energy dispersive spectrum at upper left [illegible] shows peaks at 2 3 keV ($K\alpha_1$ for S) and 2 6 keV ($K\alpha_1$ for Cl) The sulphur peak arises [illegible] the sulphated MPS and the chlorine from metal salts The peak at 8 0 keV ($K\alpha$ for Cu [illegible]) to copper in the specimen holder (Lab No 35[illegible]/78, SEM [illegible])



Fig 3

mooth globular deposit containing Ca and sulphated MPS The Kβ (3 7 keV) and the Kβ (4 0 keV) peaks of Ca are prominent The atomic marker is placed above the S-peak (Lab No 352/78 SEM 2 86)



Fig 4

Fungal hyphae (large arrow) covered with small rod like bacteria (small arrow) The [illegible] dispersive spectrum depicts phosphorus and sulphur from organic matter In [illegible] calcium was present (Lab No 1938/77 SEM 951)



Fig 5
Macroscopical view of fungi illustrating the easily detected colonies
(Lab No 1238/77 (×35))

Discussion

ndogenous contamination of contact lenses must be derived from substances issolved in the precorneal film Small molecules able to penetrate the pores of the ns polymer will only precipitate when insoluble while macromolecules (proteins PS and lipids or compounds of these) tend to cover the surface of the lens soluble calcium salts were previously considered to be the main source of ontamination Later protein coatings were regarded as the overwhelming problem nd much effort was spent in developing proteolytic enzymes to remove this npurity

In our material mucopolysaccharides (MPS) were one of the main components in e deposits MPS have also been found in a few of the previous investigations Allen et al 1978 Hilbert et al 1976 Lowther 1975 Wedler 1977) MPS in ombination with calcium (Ca) and less frequently with other elements form the ommon smooth spherical type of deposit In the few cases where MPS were the nly constituent of the deposit a microcrystalline appearance was observed by nacroscopy in high magnification and particularly in SEM

Using the less sensitive histological methods Lowther (1975) observed a crystal ike type of deposit containing calcium on lenses for day time use but only in lenses accidentally boiled in saline prepared from calcium rich artesian water However

he failed to detect calcium in the common smooth deposits on le [illegible] boiled

On one lens Allen et al (1978) found neutral MPS in smooth deposits [illegible] with Ca protein and lipids in a multilayered arrangement We have not observed such a structure on our frozen sections stained for lipids as no significant [illegible] was present

Doughman et al (1975) detected exclusively lipids and lipoproteins in [illegible] deposits on their lenses

The above mentioned differences in content of substances in the deposits [illegible] difference in type of deposit may be explained partly by the different methods [illegible] and partly by the differences of lens material Individual methods of wear [illegible] also be of importance

The assumption that proteins are a main component in lens deposits is [illegible] based on biochemical investigations (Wedler 1977) Using the far less [illegible] histological techniques we could only detect protein in two out of 99 lenses

Our examination revealed that more than two-thirds of lenses worn for [illegible] periods contained calcium According to the literature the calcium [illegible] however seems to vary and calcium can even be demonstrated in [illegible] (Allen et al 1978) Ruben et al (1975) demonstrated that Ca is deposited as [illegible] triphosphate This observation was supported by Klintworth et al (19[illegible]) [illegible] found only Ca together with phosphorus (P) in one out of eight lenses

Ca together with fungal deposits was found only in one out of three lenses [illegible] was found in most of the deposits this rare combination of fungi and Ca [illegible] indicate that fungal contamination of Ca-containing deposits is not the rule

Fungal deposits are easily detected macroscopically as dark colonies (B[illegible] 1974 Cochet 1977 Kanai et al 1977) (Fig 5) In our material bacteria were [illegible] heavy contaminant when fungi were found The bacteria were always in [illegible] contact with the hyphae of fungi suggesting a form of symbiosis This [illegible] confirmed by the fact that our study could not demonstrate any bacteria in [illegible] absence of fungi Previous studies (Doughman et al 1975 Kanai et al 19[illegible] [illegible] et al 1972) also demonstrated only a few bacteria on soft contact lenses

Evaluating the methods applied we would recommend macroscopical [illegible] tion combined with low voltage SEM of formalin vapour fixed non-coated [illegible] of lens and X ray microanalysis of the same specimen in a few cases [illegible] by LM TEM may give some structural information particularly in [illegible] bacterial deposits but the information achieved can be easier reached by the [illegible] methods

he present work indicates that the recommended methods give a useful ription of the composition of the deposits that MPS and Ca are dominant parts he deposits and that bacteria and fungi are rare and when present are always nd together

Acknowledgment

authors are indebted to several ophthalmologists for sending contact lenses with osits.

References

n J Botting R Sharp A & Tuffery A A (19 8) Deposits on contact lenses The Optician. anuary 6 8–9

wn S I Bloomfield S Pearce D B & Tragakis M (1974) Infection with the therapeutic oft lens Arch Ophthalmol 91 275–277

het P (1977) Microscopical changes in soft contact lenses Trans ophthal Soc U K 97 5 -158

ughman D I Mobilia E Drago D Havener V & Gavin M (1975) The nature of spots on soft lenses Amer J Ophthal 7 345–353

lbert J Lowther G & King J (1976) Deposition of substances within hydrophilic lenses Am J Optom & Physiol Optics 55 51–54

nai A Yamaguchi T Momose T & Nakajima A (1977) Electron microscopic studies of deposits on the soft contact lens J jap cont Lens Soc 19 19–27

intworth G K Reed J W Hawkins H K & Ingram P (1977) Calcification of soft contact lenss in patients with dry eye and elevated calcium concentration in tears I ve t Ophthalmol Vis ual Sci 16 158–161

wther G E (1975) The relationship between the chemistry of the tear film and hydrophilic lens deposits Sec Nat Res Symp on Soft Contact Lenses pp 42–50 Chicago

tas B R Spencer W H & Hayes T L (1972) Scanning electron microscopy of hydrophilic contact lenses Arch Ophthalmol 88 287–295

uben M Tripathi R. C & Winder A F (1975) Calcium deposition as a cause of spoilation of hydrophilic soft contact lenses Brit J Ophthal 59 141–148

ragakis M P Brown S I & Pearce D B (1973) Bacteriologic studies of contamination associated with soft contact lenses Amer J Ophthal 75 496–499

ripathi R C & Ruben M (1973) Degenerative changes in a soft hydrophilic contact lens An ultrastructural study Ophthal Res 4 185–192

iedler F C (1977) Analysis of biomaterials deposited on soft contact lenses J B omed Mater Res 11 525–535

k s II
r O A Jensen Øjenpatologisk Institut
ederik V s Vej 11 5 DK 2100 Copenhagen Ø Denmark

The Department of Ophthalmology (Head S Ehlers)
Århus Kommunehospital University of Århus, Århus Denmark

OPTICAL PRINCIPLES FOR ESTIMATION OF ENDOTHELIAL CELL DENSITY WITH THE NON CONTACT SPECULAR MICROSCOPE

BY

THOMAS OLSEN

In non contact specular microscopy of corneal endothelium endothelial cell density is overestimated due to the angle of observation and the curvature of cornea. On the basis of theoretical considerations it is concluded that if the angle of observation is kept at a small value the curvature of cornea contributes only insignificantly to this overestimation the major determinant being the angle of observation When an angle of 46 is chosen between slit illumination and optical axis of the microscope it is calculated that estimates of endothelial cell should be multiplied with a factor of 0 959 to correct for the angle effect. Six eyes were photographed with both a contact and non-contact specular microscope and endothelial cell density estimated Mean observed difference in cell counts was +5 5% without correction and +1 9% with correction for angle effect 95% confidence limits for the difference with correction are −3 7 and +6 0% respectively showing that estimates of endothelial cell density obtained with the non contact specular microscope agree closely with those obtained by contact specular microscopy when corrected for angle of observation

Key words endothelium – cell density – specular microscopy – cornea

Specular microscopy of corneal endothelium *in vivo* is generally performed with the technique described by Maurice (1968) This technique implies applanation of cornea with a dipping cone mounted on the objective (Laing et al. 1975 Bourne & Kaufman 1976) The applanation is of help in keeping the endothelium in f... and allowing a high magnification (about ×200)

Received January 8 1979

ie technique of visualizing the endothelium with the slit lamp was originally ribed by Vogt (1920) However it is not until recently after suggestions made r Olle Holm University of Lund Sweden that this non-contact technique has i reintroduced into clinical practice combined with a photographing device m 1978)

he non-contact specular microscope is in principle an ordinary slit lamp nted with a microscope of high magnification (about ×100) The objective of microscope has a long working distance which allows the endothelium to be rved without contact with the anterior corneal surface

he reflex from the endothelium can be dissociated from the strong reflex from corneal surface by increasing the angle of observation In contact specular roscopy the microscope objective also serves to focus the incident slit illumination cornea The angle between incident light and reflected light is with this mique very small In non-contact specular microscopy the angle is larger and y significantly disturb the dimension of the photomicrograph so that the area ered on the photograph corresponds to a greater corneal area Endothelial cell sity estimated from photographs taken with the non-contact specular micrope may therefore erronously bee too high if not corrected Another factor which y induce too high estimates of cell density is the curvature of the cornea

he purpose of this study was to provide a theoretical basis for correction of mates on endothelial cell density obtained with the non-contact specular roscope To illustrate the order of magnitude by which these factors play a nificant role estimates obtained with non-contact specular microscopy is then npared with estimates of endothelial cell density obtained with the contact cular microscope ad modum Maurice

Methods

e non-contact microscope was produced by Preisler Instrument AB Malmo eden The objective was a Leitz objective 25× magnification with a working tance of about 15 mm An Olympus OM 1 camera body was mounted directly on microscope The microscope was mounted on a metal arm designed to fit a Zeiss oto slit lamp and adjusted so that the microscope could be circled around the al point The angle between the incident light from the slit lamp and the optical s of the microscope could be kept constant (in the present study at 46°) by chanically fixing the slit lamp and the microscope to each other Photographs re taken using the Zeiss flashing device for the slit lamp and Ilford PAN F 24×36 n film, secondarily enlarged to 18×24 cm

The contact specular microscope was purchased from Syber Inc Gainesville

Florida Film Kodak TRI X 24×36 mm Secondary enlargement to [illegible] Final magnification was determined by photographing a Burger Türk [illegible] cell counting chamber

Six normal subjects (3 left and 3 right eyes) were photographed [illegible] non contact specular microscope and the contact specular microscope in [illegible] On the basis of greatest photographic clarity photographs were selected endothelial cell density was estimated from four or more pictures according [illegible] counting principle given by Gundersen (Sperling & Gundersen 19[illegible]) which [illegible] unbiased estimates of cell density to be drawn from small samples

95% confidence limits were calculated on basis of the t-distribution.

Results

Theoretical considerations

Light is always reflected from the endothelium at an angle which practically equals [illegible] of incident light Due to refraction of cornea, however the endothelium is observed [illegible] angle (u) which is smaller than the angle of incident light on the cornea (i) (Fig 1) The observation angle u is determined by

$$\frac{\sin i}{\sin u} = n$$

where n_c denotes refractive index of cornea Substituting n = 1.3 (see Davson [illegible]) i = 23 (the angle used in the present study) one gets u = 16 [illegible] If the endothelium [illegible]

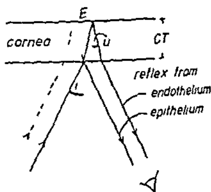


Fig 1

In non-contact specular microscopy the endothelium is seen at an angle u which is [illegible] than the angle i of incident light on cornea u is determined by $\frac{\sin i}{\sin u} = n_{cornea}$ [illegible] n_{cornea} is refractive index of cornea The maximum width E of endothelium [illegible] observed [illegible] limited by the strong reflex from the epithelium and is determined by E [illegible]

CT corneal thickness (assuming parallel beams of light)

…s this angle of observation would induce a shortening of the horizontal dimension by a … cos u assuming a horizontal angle between incident and reflected light Since the …al dimension would be unchanged this means that an area on the endothelium would …minished by a factor cos u

…e curvature of cornea however further distorts the observed dimensions In Fig 2 is …n in the horizontal plane how the angle of observation and the curvature of cornea …nce an observed area of endothelium For simplicity the outer surface of cornea has …omitted The narrow beam of slit light is reflected with maximal intensity in point P …angle of observation in this point is u In point Q the endothelium is also seen but more …y because the angle of observation is slightly greater than the angle of incident light (the …hat the endothelium is seen as a band and not as a line is due to the slight non parallel …tion of the slitlight and the non-smooth surface of the endothelium)

…PQ denotes curved true distance on cornea the observed distance PR is determined by

$$\sqrt{(r^2)(\sin^2\alpha + (1-\cos\alpha)^2)}\ \cos(\tfrac{1}{2}\alpha + u) \qquad (1)$$

…notes radius of cornea α PQ in radians and u is angle of observation

…length of PQ seen on endothelium cannot be increased beyond the limit given by the …ction from the anterior surface of the cornea due to the very intensive illumination from …surface See Fig 1 The maximal length E is determined by

$$CT\ ?\ \mathrm{tg}\,u \qquad (2)$$

…e CT is thickness of cornea

…stituting CT = 0 5 mm and u = 16 5 one gets E ~ 0 3 mm This corresponds to 0 038 …ans on cornea (α in Fig 2) assuming cornea radius to be 8 mm (r in Fig 2) Substituting …e values in *equation 1 the observed length PR of PQ (true distance 0 3 mm) becomes*

$$\sqrt{(8\,\mathrm{mm})^2(\sin^2 0\,038 + (1-\cos 0\,038)^2)}\ \cos(\tfrac{1}{2}\alpha + u)$$

$$0\,?999 \times \cos(\tfrac{1}{2}\alpha + u)$$

Fig 2

…Specular microscopy the observed distance PR corresponds to a distance PQ on cornea …ich is greater than PR depending on the angle of observation and the curvature of cornea r = radius of cornea α = PQ in radians

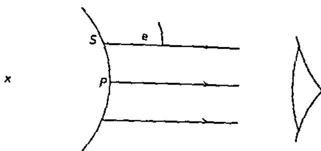


Fig 3

In non contact specular microscopy the curvature of cornea induces an [illegible] in the upper and lower part of endothelium observed when a vertical slit illumination [illegible]

and this is almost

$= PQ \times \cos(\frac{1}{2}\alpha + u)$

Substituting u = 16 5 one gets

$PR = PQ \times 0\ 9533$

If the cornea did not curve

$PR = PQ \times 0\ 9588\ (= PQ \times \cos u)$

i e the curvature of cornea contributes to less than 1% in the shortening of the [illegible] horizontal distance

With a vertical slit illumination the vertical part of the endothelium observed is [illegible] than the horizontal part With the present method the maximal vertical length of the endothelium observed is about 1 mm As seen in Fig 3 because of the curvature of the cornea this induces an observation angle (e in Fig 3) which is greatest in the upper and [illegible] part of endothelium photographed If the cell density was counted on photographs [illegible] these areas too high estimates would result The overestimation is however insignificant

If a small area in point S on Fig 3 is assumed to be 0 5 mm from [illegible] photographed this area would be observed at an angle of 0.5 [illegible] radians (or 3 6 [illegible]) length in S would consequently be shortened by a factor of cos 3 6 [illegible] insignificant error This calculation assumes no refraction of the anterior surface of cornea This refraction would ofcourse tend to diminish the error even more

These results show that if the endothelium is observed at a horizontal angle of 16° between incident light and observation the horizontal dimension is shortened by less than [illegible] per cent The curvature of cornea contributes only insignificantly to this shortening The [illegible] dimension is almost unchanged due to the insignificant curving of cornea in the [illegible] observed It is therefore possible to correct an area observed with a reasonable [illegible] namely with the factor given in equation 4

Non contact and contact specular microscopy of corneal endothelium

In Table I the results of estimates of endothelial cell density obtained [illegible] non contact as well as contact specular microscopy are given The photographs [illegible] the non contact specular microscope (Fig 4) are taken at an angle of [illegible] light This corresponds to 16 5 observation angle of endothelium (Fig 1) [illegible]

if no correction is made for the angle of observation the cell density is estimated compared to the estimates obtained with the contact specular oscope The cell densities obtained with the latter can be regarded as true ues due to the very small angle of observation From the distorsion on ographs of the squared micro-scale of Burger Turk this angle was calculated to ss than 6 i e overestimating cell counts by less than 1 per cent

the correction factor outlined in equation 4 (= 0 959) is applied on the nates obtained with the non-contact specular microscope the mean difference ell counts diminishes from + 5 5 to + 1 2 % making the estimates almost tical 95 % confidence limits for the mean difference with correction are − 3 7 + 6 0 % respectively

Conclusion

s study has shown that if not corrected endothelial cell density is overestimated 1 the non-contact specular microscope The major determinant for this over mation is the angle of observation while the curvature of cornea contributes

Table I

nates of endothelial cell density (cells mm²) obtained with contact and non-contact specular microscopy (sp m) with and without correction for angle of observation (mean ± SEM)

ient	Contact sp m	Non-contact sp m		Difference (%)	
		Without correction	With correction (× 0 959)	Without correction	With correction
TB	2863 ± 69 3 (n = 9)	3031 ± 37 1 (n = 5)	2907 ± 35 6	+ 5 9	+ 1 5
JC	2191 ± 79 7 (n = 12)	2341 ± 43 1 (n = 7)	2245 ± 41 2	+ 6 8	+ 2 5
HK	2887 ± 89 9 (n = 11)	3113 ± 27 8 (n = 6)	2986 ± 26 5	+ 7 8	+ 3 4
JJ	1 65 ± 64 5 (n = 6)	1825 ± 55 9 (n = 8)	1750 ± 53 4	+ 3 4	− 0 8
OH	3390 ± 84 5 (n = 4)	3529 ± 49 7 (n = 9)	3384 ± 47 7	+ 6 3	+ 1 9
AJ	2770 ± 59 1 (n = 10)	2846 ± 69 3 (n = 5)	2739 ± 66 3	+ 2 9	− 1 4

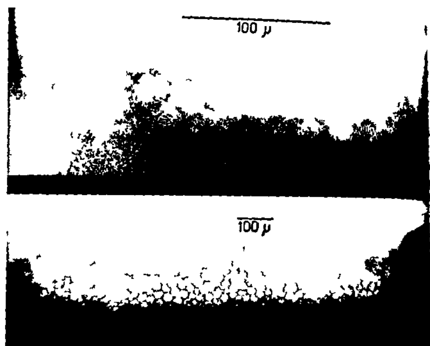


Fig 4
Normal corneal endothelium photographed *in vivo* with a contact and a non-contact sp... microscope The largest field of observation is seen with the non-contact specular mi...

only insignificantly and may be neglected if the angle of observation is kept at a small value (in the present study at 23) When corrected for this angle effe... estimates agree closely with those obtained by contact specular microscopy

Acknowledgment

This work was supported by grants from Fonden til Lægevidenskabens fremme The technical assistance of Mrs Anette Poulsen is gratefully acknowledged

References

Bourne W M Kaufman H E (1976) Specular microscopy of human corneal endothelium in vivo *Amer J Ophthal 81* 319–323
Davson H (1969) *The Eye* Academic Press New York and London
Holm O (1978) High magnification photography of the anterior segment of the ... Trans sweed ophthal soc 1977 *Acta ophthal (Kbh) 56* 4...

R. A Sandstrom M M Leibowitz H M (197ɔ) *In vivo* photomicrography of the ... eal endothelium A ch. Ophthal 93 143–14ɔ

... ce D M (1968) Cellular membrane activity in the corneal endothelium of the intact ... *Experientia* 24 1094–109ɔ

... g S & Gundersen H J G (1978) The precision of unbiased estimates of numerical ... sity of endothelial cells in donor corneas *Acta ophthal (Kbh)* 56 793–802

... L (1920) Die Sichtbarkeit des lebenden Hornhautendothels ein Beitrag zur Methodik ... Spaltlampenmikroskopie *Gra fes Arch* 10 123–144

... s address

... of Ophthalmology

... unehospital

Arhus C, Denmark

Department of Ophthalmology (Head Salme Vannas)
University of Helsinki Helsinki Finland

EFFECTS OF CORNEAL PRESERVATION DONOR AGE CADAVER TIME AND POSTOPERATIVE PERIOD ON THE GRAFT ENDOTHELIUM

A specular microscopic study

BY

PEKKA RUUSUVAARA

The endothelia of 102 clear human corneal grafts in 95 patients were photographed *in vivo* with a specular microscope The endothelial cell densities of the grafts were counted from an area of 0 01 mm² The follow up period averaged three years The patients ages ranged from 16 to [illegible]7 years and that of the donors from 8 to 89 years The average endothelial cell density for all [illegible] transplants was 1169 ± 478 cells/mm²

Corneal transplants stored in MK medium (N = 19) have more cells than cryopreserved grafts (N = 42) or grafts stored in the moist chamber (N = 41 this last group was used as control material The differences in cell densities were statistically significant The cell density of cryopreserved grafts was significantly higher than in grafts stored in the moist chamber

Only in those grafts in which moist chamber storage had been used was endothelial cell density dependent on time of storage With an increase in cadaver time (time from death to enucleation) the endothelial cell density of the graft decreased

An inverse correlation was found between the cell density of the graft and donor age Good results were still found with healthy donors over 60 years The endothelial cell density of the grafts showed a steady decrease during the long term post-operative period the rate of cell loss being much higher in graft endothelium than in normal endothelium during ageing

Key words cornea corneal graft endothelium corneal preservation – specular microscopy – cell viability – donor corneal material – cryobiology

Received January 22 1979

a_e of donor corneas has al ays been a major problem in corneal grafting (M as 1939) Filatov started the use of cadaver eyes in the 1930 s Since that time reservation of the cadaver eye or the cornea alone has become increasingly rtant Nowadays we need a well preserved cornea with potential function for s a penetrating graft A good criterion of adequate preservation of the cornea intact functioning corneal endothelium (Breslin & Ng 1976)

be successful a corneal graft has to have optical clarity regular curvature and mally functioning endothelium The corneal endothelium functions firstly as a er and secondly as a metabolic dehydrating pump (Kaufman et al 1966 Kaye 1973) The success of the graft depends chiefly on whether the preserved or cornea has a viable endothelium The adult human endothelium is believed o regenerate at all and when damaged the endothelial cells heal by spreading so covering the area of the dead cells (Bourne & Kaufman 1976b)

1968 Maurice described a specular microscope for studying the endothelium e intact cornea *in vivo* This instrument has subsequently been modified by g et al (1975) and by Bourne & Kaufman (1976a) This *in vivo* photomicro- hy affords an exact method for measuring the density of endothelial cells after toplasty (Bourne & Enoch 1976 Sherrard 1976 1977)

udies made with the specular microscope have shown that corneal transplants fewer central endothelial cells than normal corneas (Bron & Brown 1974 rne & Kaufman 1976b) The endothelial cell density of the graft does not ear to be significantly correlated with corneal thickness rejection episodes the nosis of the recipient or the method of donor tissue preservation (Sato 1978) ever corneas transplanted from younger donors to younger recipients have a ificantly greater number of central endothelial cells (Bourne & Kaufman 6b) The results of previous studies on the significance of the different ameters of corneal grafting may give an unrepresentative picture because of the ll numbers of patients and short follow up periods

The average endothelial cell densities in corneal grafts show a wide variation m one series to another (Bron & Brown 1974 Bourne & Kaufman 1976b) The test density of endothelial cells was reported by Bourne et al (1977) in gan-cultured corneas with an average cell density of 1548 cells mm²

Opinions still vary about the best methods for preservation of corneal buttons or globes and also about the critical preservation time and donor s age after which cornea is no longer suitable for penetrating keratoplasty With cryopreserved terial it has been calculated or recommended that the donors should be under e age of 50 years and the cornea must be excised within six hours of the death of e donor (King 1970) However a fresh cornea or material used after moist amber storage is still preferred to preserved material because the transplanted dothelium is assumed to have a better viability

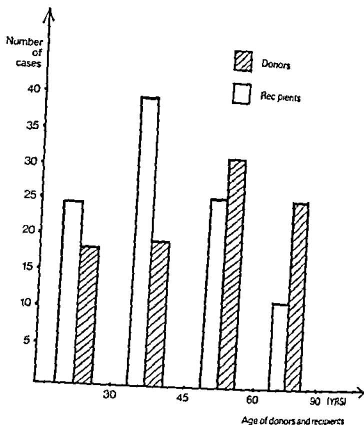


Fig 1
Age distribution of donors and recipients.

This paper reports the results of measurements of endothelial cell densities in [illegible] clear human corneal transplants made at the Helsinki University Eye Hospital. The first point studied was the extent to which the endothelial cell density of the [illegible] depended on the method of preservation. In addition the importance of donor age, cadaver time (time from death to enucleation or excision of the cornea), preservation time and some other parameters were evaluated. The subsequent [illegible] of the graft endothelium was also elucidated.

Patients and Methods

Patients

The clinical series comprised 102 penetrating keratoplasties in 9[illegible] patients. Of the graftings, seven were bilateral and three were regraftings. This follow up [illegible] made during the years 1976–78. The follow up period varied from 1 month [illegible]

s on average 3 years The patients ages ranged from 16 to 77 years and that of donors from 8 to 89 years (Fig 1) Among the patients the ratio of men to ien was 55 to 40

cal diagnosis of the patients

re were altogether 60 eyes with keratoconus and 8 patients had other corneal rophies The corneal opacities were due to herpes tuberculosis or lues in 7 3 9 cases respectively in 11 patients the corneal inflammation had some other se and in 4 patients the cause was trauma or unknown

rative methods and postoperative treatment

operations were performed mostly by the same two surgeons (SV and KK) size of the graft varied from seven to eight mm The graft was cut from the ior globe with Franceschetti s trephine or from the corneal button with Polack ch (Brightbill et al 1973)

During *the last six years a microsurgical unit (Moller Wedel) has been used* tead of the previous magnifying loupe Before the operation the condition of the ift was checked with the microscope and after punching the graft the peripheral neal rim was examined by vital staining of the endothelium

As suture material a 10–0 nylon monofilament was used for continuous or single ures In the earliest cases 9–0 virgin silk sutures were used

As prophylaxis all patients received topical or peroral corticosteroid and when cornea was heavily vascularized the patient was also treated with immuno-ppressives

thods of corneal preservation

he patients were *grouped according to the method of preservation of the donor* rneas The first group which was used as control comprised patients with grafts ored in a moist chamber as whole globes at 4 C for less than 48 h Of these 16 were esh, which means that the storage time was less than one hour The total number f transplants in this group was 41 It is recommended that the time interval etween enucleation and keratoplasty should be less than 48 h (Paton 1955)

The second group comprised patients who had a cornea after intermediate term reservation in the M K medium devised by McCarey & Kaufman (1974) The donor cornea had been excised with a Draeger s electric rotor trephine within 11 h of death (S Vannas 1975) *In this method the preservation time varied from one to* four days in tissue culture medium at 4 C McCarey & Kaufman s recommendation for critical storage time with this method is up to five days

The third group comprised grafts which had been cryopreserved according to

Table I

Endothelial cell density and preservation methods (± s.)

Preservation method	Number of transplants	Mean age of donors (years)	Mean age of recipients (years)	[illegible]
Cryopreservation	42	(N = 41) 51.4	40.0	[illegible]
Storage in M-K medium	19	(N = 19) 46.2	44.9	[illegible]
Moist chamber storage	41	(N = 36) 47.9	40.1	[illegible]
Total	102	(N = 96) 49.1	40.8	[illegible]

Table II

Endothelial cell density in transplants to patients with keratoconus and other diseases after different types of preservation

Preservation Method	Diagnosis of patients	Number of transplants	Mean age of donors (years)	Mean age of recipients (years)	[illegible]
Cryopreservation	conus	27	(N = 26) 54.3	33.9	[illegible]
	others	15	46.5	59.3	[illegible]
Storage in M-K medium	conus	7	35.4	31.1	[illegible]
	others	12	59.4	51.8	[illegible]
Moist chamber storage	conus	26	(N = 22) 45.8	31.9	[illegible]
	others	15	(N = 14) 51.4	60.9	[illegible]
Total	conus	60	(N = 55) 49.5	33.3	[illegible]
	others	42	(N = 41) 49.9	51.4	[illegible]

the method of Capella et al. (1965) and Kaufman & Capella (1968). All corneas had been excised and placed in storage within six hours post mortem. The time limit [illegible] this method has been estimated at two years. In this group the frozen corneas had been in the eye bank from one day to three years. The total number of cryopreserved corneas was 42.

The average ages of the recipients and the donors in different preservation groups are seen in Tables I & II.

Table III
Donor age and endothelial cell density

Age group of donors (years)	Total number of transplants	Mean age of donors	Mean age of recipients	Endothelial cell density (cells/mm²)
8–30	18	22 9	35 2	1424 ± 597
31–45	20	37 8	42 2	1401 ± 592
46–60	32	52 3	45 6	1105 ± 410
61–70	12	66 8	39 2	922 ± 253
>70	14	76 3	37 0	1011 ± 285
Donor not known	6		38 2	842 ± 359
Total	102	49 1 (96)	40 8	1169 ± 4 8

nor age

o evaluate the influence of donor age on graft endothelial cell density the patients re divided into four groups according to the age of the donor In the first group e donors were ≦ 30 years whereas in the last two groups the donor ages were tween 61 and 70 and over 70 years In six cases donor age was unknown (Table I).

otography of the corneal endothelium

he endothelium of the clear transplant was photographed with a clinical specular icroscope (Seyber Inc) The image on the negative was × 100 This was checked photographing a calibrated glass slide scored at 10 μm intervals The prints had a nal magnification of exactly × 500 (Fig 2a b c) The endothelial cell density uld be counted from the photographs by counting the number of cells in a known ea which corresponded to 0 01 mm² in nature In most cases three to five central raft cell areas were counted and the average was taken as the result

istical analyses were made by Student s *t* test to ascertain the effects of various factors on e parameters studied

The patients were divided into different groups so that enough cases were found in each of em to make the statistical analyses possible Also the different groups of cases were made so at the recommended or suggested critical times or ages could be checked and compared th the other poss bilities for example critical cadaver time postoperati e period or donor ge. In the text and tables the numeral values of cell densities ± standard deviations are to be een.

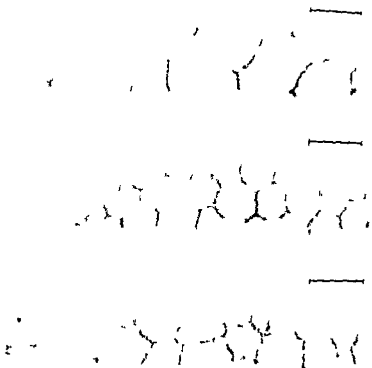


Fig 2

A Central corneal endothelium on specular microscopy of a graft stored in moist chamber for three hours The mean cell density is 650 cells mm² (× 500) Bar 50μ

B Central corneal endothelium of the graft stored in M K medium for three days The mean cell density is 2025 cells/mm² (× 500) Bar 50μ

C Central corneal endothelium of a cryopreserved graft The preservation time was years The mean cell density is 1425 cells/mm² (× 500) Bar 50μ

Results

Effect of corneal preservation on endothelial cell density (Tables I & II)

A total number of 102 clear penetrating transplants in 95 patients were photographed with the specular microscope The average endothelial cell count for all 102 transplants was 1169 ± 478 cells/mm² with a mean donor age of 49 1 years

The patients were divided into three different groups according to the method by which the donor corneas had been stored In the control group with grafts

sh or after moist chamber storage the mean endothelial cell density was 4 ± 390 cells/mm² the values ranging from 500 to 1850 cells/mm² The number transplants was 41 The average recipient age was 40 1 and the average donor e 47 9 years

In the grafts stored in M K medium the mean endothelial cell density was 4 ± 456 cells/mm² the values ranging from 1000 to 2700 cells/mm² In this oup there were 19 patients The average recipient age was 44 2 years and the erage donor age 46 2 years

Among the cryopreserved grafts the mean endothelial cell count was 1102 ± 308 ls mm² the values ranging from 400 to 2100 cells/mm² This group contained 42 tients The average recipient age was 40 0 years and the average donor age 51 4 ars.

Corneal transplants stored in M K medium have more cells than cryopreserved afts ($P < 0.001$) or grafts stored in a moist chamber ($P < 0.001$) The difference endothelial cell density after cryopreservation and after moist chamber storage as also statistically significant ($P < 0.001$)

The statistical significance of the differences in cell counts is even clearer when ly the 60 keratoconus cases are considered (Table II)

reservation time

n cryopreserved material the preservation times varied from one day to three years nd two weeks in material stored in M K medium from one to four days and in resh corneas or material stored in a moist chamber from 0 to 48 h Each group was vided into subgroups according to preservation time No statistically significant ifference could be detected between the different preservation times within roups of M K medium stored or cryopreserved grafts

However the group with a moist chamber time of less than one hour (fresh orneas) was found to differ statistically from the others ($P < 0.01$) No really ritical moist chamber time was observed to exist in the time interval from 1 to 48 h

ect of donor age

Table III shows the mean endothelial cell densities in the different donor age groups The results were best in the two youngest groups with donors aged 45 years or less In the first group with donors aged 8 to 30 years the endothelial cell count was 1424 ± 527/mm² When the oldest donors were divided into two groups on both sides of 70 years there was no statistically significant difference between them

An inverse correlation was found between the cell density of the graft and donor age the difference being statistically significant between donors younger and older than 45 years ($P < 0.01$)

Table IV
Effect of cadaver time (time from death to enucleation or excision) on graft endothelial cell density

Cadaver time (hours)	Number of transplants	Mean age of donors	Mean age of recipients	Endothelial cell density
0– 1	33	(N = 31) 46.8	40.8	[illegible]
1– 4	22	(N = 20) 50.5	41.[illegible]	[illegible]
4– 6	22	47.5	[illegible]	1099 [illegible]
6–10	13	54.8	49.5	1030 ± [illegible]
10–20	8	(N = 7) 43.1	[illegible]	[illegible]
Unknown	4	(N = 3) 49.3	46.3	1113 ± [illegible]

Effect of cadaver time (Table IV)

The endothelial cell density of the graft decreased with increase of cadaver time (time from death to enucleation). Much the best results were obtained when cadaver time was less than one hour. This group differed almost significantly ($P < 0.05$) from the others, and when the cadaver time was more than 10 h the difference was statistically significant ($P < 0.01$).

The mean cadaver times for grafts stored by different methods were all between 2½–6 h.

Effect of postoperative period (Table V)

The cell density showed a steady decrease with postoperative period. A statistically significant difference ($P < 0.001$) in cell density was noticed if the postoperative [illegible]

Table V
Correlation between postoperative period and endothelial cell density of the graft.

Postoperative period (years)	Number of transplants	Mean age of donors	Mean age of recipients (years)	Cell density (cells/mm²)
≦ ½	16	53.0	38.1	1513 ± [illegible]
½– 1	19	47.1	[illegible]	[illegible]
1– 2	16	41.9	41.9	1311 ± [illegible]
2–	2[illegible]	(N = 2[illegible]) [illegible]	43.0	[illegible]
[illegible]–11	26	(N = [illegible]) 48.7	[illegible]	[illegible]

od was more than two years as compared with the shorter postoperative times rate of cell loss was much higher with graft endothelium than with normal eal endothelium during ageing

he follow up period for the grafts stored in M K medium averaged *one year* opreserved grafts had a mean follow up period of two years and for moist mber stored grafts it was 5½ years

Discussion

mean endothelial cell density for all 102 transplants was 1169 ± 478 cells/mm² is corresponds well to the results of other authors for example Bourne & ufman (1976b) found a mean cell density of 973 cells/mm² and Bron & Brown 4) a mean cell density *of 1125 cells/mm² Thus far the highest cell density* 18 cells/mm² has been found in the series of Bourne et al (1977) of 14 an-cultured transplants In their study the follow up period was quite short and *can only* guess at the long term values *All* studies concerning corneal endo- lial cell density indicate that we lose quite a large number of endothelial cells ing preservation and transplantation In my series with donors whose average was 49 1 years I found a mean endothelial cell loss of rather more than 50% as pared to the endothelial cell density of the normal cornea

The data of this investigation support the view that one of the best methods of serving corneal endothelium for keratoplasty is intermediate term corneal age in McCarey Kaufman (M K) medium With preservation in M K medium transplants had *many more endothelial cells (1847 ± 456 cells/mm²) than with* two other methods used in our hospital moist chamber storage (924 ± 320 ls mm²) and cryopreservation (1102 ± 308 cells/mm²) The average follow up iod was *three* years which is supposed to be long enough for manifestation of l loss due to preservation and operation The average donor age in the different servation groups did not differ significantly So donor age cannot be the reason r the variation in endothelial cell densities

Before the advent of cryopreservation and intermediate term preservation of meas it was believed that material used fresh or after moist chamber storage uld give the best corneal grafts However McCarey & Kaufman (1974) showed electronmicroscopy and the temperature reversal viability test that storage of cised corneas in M K *medium gives better results than storage of fresh corneas* whole globes in a moist chamber Our study confirms that after moist chamber orage transplants have significantly fewer endothelial cells than after storage in 1 K medium

Our new finding was that the mean cell density of grafts was significantly [illegible] after cryopreservation than after moist chamber storage. Presumably this [illegible] on the difference in environment, the corneal endothelium being [illegible] poisoned by degradation products of tissues when stored in a moist chamber [illegible] the cornea is trephined from the globe (Schimmelpfennig et al. [illegible]). There [illegible] little fluid in the anterior chamber and metabolic degradation products [illegible] accumulate there soon after the donor's death. According to our results [illegible] corneal excision during the first hours postmortem is of the utmost importance.

The mean follow up period for grafts stored in moist chamber was [illegible] grafts which were cryopreserved. This fact may in some degree [illegible] difference in cell densities between these two groups, but does not [illegible] difference. The loss of endothelial cells after two years was very small (Table [illegible]) and, on the other hand, the mean follow up period for cryopreserved grafts was [illegible] two years.

Even a relatively small number of endothelial cells can keep the cornea [illegible] of normal thickness. This was observed in the transplants made after moist chamber storage and cryopreservation. With the M-K method we were able to [illegible] nearly twice as many endothelial cells as with the other two methods. In that respect intermediate term corneal storage using M-K medium seems superior to the [illegible] alternative methods.

A very interesting finding is that with cryopreserved material our transplants [illegible] more endothelial cells than those used after moist chamber storage, the difference being statistically significant. Thus the cryopreservation method appears to [illegible] the best possibility of corneal storage for long enough to allow selection [illegible] HLA compatible donor recipient pairs. This increases the chances of successful corneal grafts in highly vascularized and so called bad risk patients (S[illegible] et al. 1976). Whether HLA compatibility affects endothelial cell density was [illegible] discussed in a further paper.

The cadaver time (time from death to enucleation or excision) was noted [illegible] influence the endothelial cell density of the grafts. Statistical analysis [illegible] correlation between cadaver time and cell density showed that when the cornea was excised in less than one hour, the cell density was significantly higher than in the other cadaver time groups. Further, when cadaver time exceeded 10 h, the cell density was significantly less than in the other groups. So far it has been [illegible] that 6 h is the critical cadaver time, but in this study I found that we can [illegible] obtain a good result up to 10 h post mortem. This is one reason for the better [illegible] density in cryopreserved material compared with material stored in a [illegible] chamber, that is, the shorter cadaver time for cryopreserved material, which [illegible] hospital has been 6 h.

An important factor for endothelial cell survival in corneal grafts seems to [illegible]

of the donor The highest cell density was found in grafts from donors under ears of age (over 1400 cells/mm²) Bourne & Kaufman (1976b) likewise found nverse correlation between donor age and the central endothelial cell density of graft, and came to the conclusion that the younger the donor and the younger recipient the more numerous will be the central endothelial cells

ne of the most important point that emerged in this analysis of the correlation ween endothelial cell density and donor age was that after the age of 45 age es little or no difference Among the older donor groups healthy material even n donors over 70 years-old gave results that were nearly as good as judged by othelial cell density as corneas from donors in the age group 46–60 years The density of 1011 ± 285 for donors of over 70 years is as good as the mean cell sity of around 1000 cells/mm² in many reports (Bron & Brown 1974 Bourne & ufman 1976b Sato 1978)

he follow up period varied from 1 month to 11 years It had a significant effect the endothelial cell density of the graft The cell density was highest in the nsplants seen only six months after the operation A statistically highly significant erence in the endothelial cell density of the graft was found two years toperatively It is known that endothelial cell densities decrease with age urne & Kaufman 1976a Laing et al 1976a b) but this analysis of the graft lothelium showed that postoperatively it loses cells at a much greater rate than normal endothelium All this suggests that transplanted endothelium will be ch more vulnerable to postoperative complications such as glaucoma trauma l other eye diseases (Jones & Rice 1967) It also underlines the importance of n planting as many endothelial cells as possible even though for some time after ration the cornea can be kept clear with a very small number of endothelial cells about 13–15% of the normal cell density

n this study the importance of three different preservation methods was luated Long term cryopreservation was found to preserve more endothelial ls than moist chamber storage of eye globes however M K medium storage was erior to both the other methods The long follow up also showed the impor ce of short cadaver time donor age and the vulnerability of transplanted dothelial cells The influence of histocompatibility on graft endothelial cell nsity remains to be studied

References

Bourne W M Doughman D J & Lindstrom R L (19 7) Organ [illegible] endothelium *in vivo* *Arch Ophthal 95* 1818–1819

Bourne W M & Enoch J M (1976) Some optical principles of the [illegible] microscope *Invest Ophthal 15* 29–32

Bourne W M & Kaufman H E (1976a) Specular microscopy of human corneal endothelium [illegible] *in vivo* *Amer J Ophthal 81* 319–325

Bourne W M & Kaufman H E (1976b) The endothelium of clear corneal [illegible] *Ophthal (Chicago) 94* 1730–1732

Breslin C W & Ng W (1976) The endothelial function of donor corneae [illegible] enucleation and refrigeration *Invest Ophthal 15* 73?–739

Brightbill F S Polack F M & Slippey T (1973) A comparison of two [illegible] donor corneal buttons *Amer J Ophthal 75* 500–506

Bron A J & Brown N A P (1977) Endothelium of the corneal graft *Trans* [illegible] *U K 94* 863–873

Capella J A Kaufman H E & Robbins J E (1965) Preservation of viable corneal [illegible] *Arch Ophthal (Chicago) 74* 669–673

Filatov V P (1937) Transplantation of the cornea from preserved cadaver eyes *Lancet* 1395–1397

Jones B R & Rice N S C (1967) The avoidance of damage to the corneal [illegible] penetrating keratoplasty I Operative techniques 9 Experimental [illegible] Rycroft P V ed *Corneoplastic Surgery Proc 2nd Int Corneoplastic Conf* London [illegible] 307–314 Pergamon Press London

Kaufman H E Capella J A & Robbins J F (1966) The human corneal [illegible] *J Ophthal 61* 835–841

Kaufman H E & Capella J A (1968) Preserved corneal tissue for [illegible] *Cryosurg 1* 125–129

Kaye G I Sibley R C & Hoefle F B (1973) Recent studies on the nature and [illegible] corneal endothelial barrier *Exp Eye Res 15* 585–613

King J H Jr (1970) Donor eyes and eye banks *Int Ophthal Clin 10* 313–3[illegible]

Laing R A Sandstrom M M Berrospi A R & Leibowitz H M (19[illegible]) [illegible] changes in corneal endothelial cells after penetrating keratoplasty *Amer J* [illegible] 459–464

Laing R A Sandstrom M M Berrospi A R & Leibowitz H M (19[illegible]b) [illegible] corneal endothelium as a function of age *Exp Eye Res 22* 58?–594

Laing R A Sandstrom M M & Leibowitz H M (1975) *In vivo* photomicrography of the corneal endothelium *Arch Ophthal (Chicago) 93* 143–145

McCarey B E & Kaufman H E (1974) Improved corneal storage [illegible] 165–173

Maurice D M (1968) Cellular membrane activity in the corneal endothelium of the intact eye *Experientia (Basel) 24* 1094–1095

Paton R T (1955) *Keratoplasty* pp 113–136 McGraw Hill Book Co New York

Sato T (1978) Studies on the endothelium of the corneal graft *Jap J Ophthal* [illegible]

Schimmelpfennig B Bigar F Witmer R & Hurzeler R (1976) Endothelial [illegible] human donor corneas *Albrecht v Graefes Arch klin exp Ophthal 200* 201–210

Sherrard E S (1976) The corneal endothelium *in vivo* its response to mild trauma *Exp Eye Res 22* 347–357

rard E S (1977) Specular micros opy of the corneal endothelium *Ophthal Opt 1*˜ 9–113
as M (1939) Beitrage zur Transplantation der Hornhaut mit einigen Vorschlagen zur rbesserung der Operationstechnik. *Albrecht v Graefes Arch Ophthal 140* 709–724
as S (19/5) Excision of the donor cornea instead of enucleation *In e t Ophthal 14* 3–995
as S Karjalainen K Ruusuvaara P & Tiilikainen A (1976) HLA-compatible donor rnea for prevention of allograft reactions *Albr cht v C aefes Arch klin exp Ophthhal 198* /– 99

or address

a Ruusuvaara M D The University Eye Clinic
versity of Helsinki SF 00290 Helsinki 29 Finland

Department of Ophthalmology (Head [illegible]) Århus Kommunehospital University of Århus Denmark

THE SPECULAR MICROSCOPIC APPEARANCE OF CORNEAL GRAFT ENDOTHELIUM DURING AN ACUTE REJECTION EPISODE

A Case Report

BY

THOMAS OLSEN

The specular microscopic appearance of a graft endothelium during a successfully treated acute endothelial rejection episode is reported. [illegible] with the increased thickness of the graft great disturbance in cellular morphology was observed. Individual cells were seen to appear swollen or to [illegible] bright intracellular areas. Also abnormalities with respect to cellular [illegible]tions were seen with the occurrence of junctional complexes at which more than three cells met. The significance of these abnormalities is discussed. The morphological changes subsided gradually as the graft cleared and the thickness decreased. This study examplifies the value of the [illegible] specular microscopic technique which enables a thorough study of the diseased corneal endothelium without traumatizing the cornea and interfering [illegible] subsequent specular microscopic examinations.

Key words: specular microscopy – cornea – endothelium – rejection – graft.

The appearance of corneal endothelium in response to trauma has been [illegible] by means of specular microscopy following various forms of insult such as [illegible] anterior chamber (Leibowitz et al. 1974), osmotic dehydration of the [illegible] (Sherrard 1976, 1978) and ultrasound (Olson et al. 1978). Most of these [illegible] have been carried out on animals, little information is available on the [illegible] endothelium which may apply to the clinical situation.

The fact that *in vivo* specular microscopy of corneal endothelium [illegible] translucent cornea hinders to a certain extent a detailed study of [illegible]

Received February 7, 1979

um following trauma The case presented here is an exception to that rule in ...it was possible to study the specular microscopic appearance of a corneal graft othelium shortly after onset of an acute rejection episode

Methods

ral corneal thickness was measured with a modified Haag Streit pachometer (Sperling & s 1977) Specular microscopy was performed using a non-contact technique described er (Olsen 1979)

presentation

30-year-old man had penetrating keratoplasty done for the first time in 1974 due to a ularized corneal scar after herpetic keratitis The grafting was successful until one year where the patient experienced an acute rejection Two weeks passed before he attended ontrol In spite of topical and systemic steroid treatment the graft remained oedematous became vascularized Retransplantation was undertaken January 1977 Postoperatively graft was resutured in the lower left quadrant but otherwise the postoperati e course was ventful. The case was included as No 1 in the study of Ehlers & Bramsen (1978)

n November 7th 1978 the patient awaked in the morning with blurred vision On mination the same day the graft was oedematous with epithelial bullae and scattered cipitates on the posterior aspect of graft No rejection line was present on this first day f onset but the cornea was thicker in the lower part than in the upper part Systemic ment with prednison 30 mg daily topical ultracortenol and subconjunctival decadrone phate injections were started immediately On this treatment graft thickness decreased

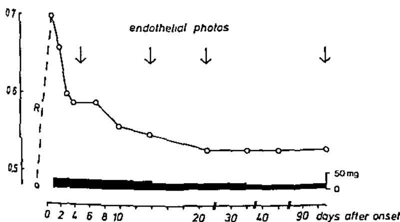


Fig 1

Diagram showing changes in central corneal thickness (CCT) (mm) of graft during an acute rejection episode Black horizontal staircase line indicates systemic prednisone treatment

gradually (Fig 1) On the 3rd day after onset of symptoms a typical [illegible] across the lower part of graft This line was later broken up into smaller [illegible] the line the cornea was constantly thicker than above the line The [illegible] in the ensuing weeks in which the graft gradually cleared Ten days [illegible] precipitates were found with the slit lamp

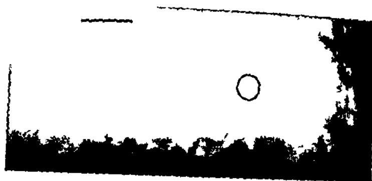






Fig 2

Endothelial cells of graft visible in the specular microscope 5 days after onset of [illegible] Top photo from upper part of graft Centre and bottom photos from [illegible] more heavily attacked part of graft The endothelial morphology is severely [illegible] swelling of cells (examples shown by arrows) intracellular bright areas [illegible] abnormal cellular junctional complexes (circles) Horizontal bar = [illegible]

Specular microscopy

he first 4 days after onset of rejection episode it was not possible to observe and ograph the endothelium due to opacification of the graft.

after onset (Fig 2)

he endothelial cells became discernible At places no planar reflex from the othelium was obtainable probably due to great distortion of endothelium In e places in which the cells were visible some cells showed dark round cellular areas which made the cells appear swollen as interpreted from the nt reflex from the center of the cells (arrows in Fig 2) Such cells also appeared round than cells without this phenomenon Other cells showed intracellular tion with respect to intensity of reflected light giving the impression of bright cellular areas (asterixes in Fig 2) Cells were larger and more irregular in the r than in the upper part of cornea

bnormalities were also found with respect to cellular junctions Normally cell ndaries meet each other at points where three cells meet Cell meetings lving four or more cells are practically never seen in normal endothelia (Fig 6) ome places cells were found to meet four at a time (circles in Fig 2) thereby dly disturbing the regular hexagonal pattern

day after onset (Fig 3)

raft almost clear The endothelial cells now reflected the light in a more even tion. Intracellular round dark areas were less pronounced but still intracellular areas were present In Fig 3 abnormal cellular intersections are especially ent showing cell meetings involving more than three cells (circles in Fig 3)



Fig 3

ndothelium of graft 14 days after onset of rejection Photograph from lower part of graft ares indicate abnormal cellular junctions with dark spots occurring in the centre Other symbols explained in Fig 2 Bar = 100 µm



Fig 4

[illegible]he specular microscopic appearance of graft 21 days after onset of rejection [illegible] [illegible]graph from lower part of graft. Size and shape of cells show considerable variation [illegible] cells with convex borders (arrows) appearing between larger cells. Bar = 1[illegible]





Fig 5

93 days after onset of rejection the endothelium has reorganized into a more regular [illegible] Top upper part of graft Bottom lower part of graft Note absence of previous [illegible] difference in cell size from top to bottom of graft. Overall cell density is about [illegible] which is clearly much lower than on previous photomicrographs. Bar 10[illegible]

.ly these abnormal junctional complexes consisted of joints of four cells but in 3 a junctional complex of five cells is also found (circle in right part of Fig 3) cell pattern is far from the normal hexagonal regular pattern the morpholo- changes being more pronounced in the lower than in the upper half of cornea.

day after onset (Fig 4)

graft now appeared clear Cell size and shape showed still considerable ation with small cells with convex borders (arrows in Fig 4) appearing between er irregular cells Cell meetings where more than three cells met were only sionally encountered (circle in Fig 4)

day after onset (Fig 5)

raft thickness had now stabilized at a value somewhat above the thickness before onset of rejection. Size and shape of cells were now more uniform but not pletely regular Whereas in the foregoing specular microscopic examinations

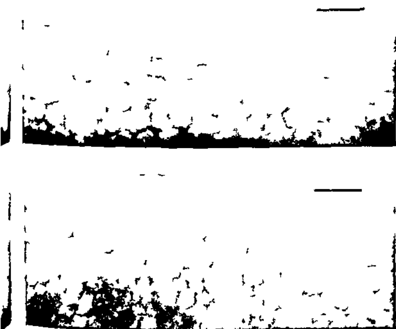


Fig 6

specular microscopic appearance of normal corneal graft endothelium Top male 69 dothelial density 1090 cells mm² Bottom female 48 endothelial density 1910 cells mm² Bar indicates 100 µm

there was a considerable difference in size of cells from [illegible] and lower part of graft this difference now was less apparent. The [illegible] of cells per unit area at this time was clearly much lower than that of [illegible] endothelium (Fig 6) and that of the first specular microscopic [illegible] was difficult to give figures for this difference due to the large [illegible] density observed within the graft No clearcut abnormalities [illegible] respect to cellular intersections were found

Discussion

In interpreting the endothelial changes observed in this study it is [illegible] to regard them as partly due to the nature of the actual disease and partly [illegible] secondary healing mechanisms of the endothelium Graft rejection [illegible] believed to be a cell mediated immunological manifestation With the [illegible] microscope it was not possible to observe any lymphocytes on the [illegible] although precipitates were seen with the slit lamp It was remarkable [illegible] the cellular abnormalities in the endothelium were so widely dispersed [illegible] occurred at places without precipitates Furthermore the rejection [illegible] the slit lamp did not sharply divide the endothelium into an abnormal and [illegible] area These non localized changes may be the result of soluble factors [illegible] the cellular hypersensitivity reaction for which a number of mediators [illegible] known to be involved (see David 1973) among them macrophage [illegible] and factors cytotoxic for target cells Some of these factors may [illegible] enzymes Interestingly this process has been shown to result in [illegible] rounding of target cells and their organelles followed by lysis of the cell [illegible] 1970) This may be the explanation for the apparent swelling of [illegible] observed in the present study (Fig 2)

The observed bright intracellular areas observed in the present [illegible] certain resemblance to those events described in rabbits (Sherrard [illegible] Leibowitz 1974) after air or osmotic dehydration of cornea. Sherrard [illegible] some of these events may progress to cell death after which a characteristic [illegible] formed by adjacent cells spreading to cover the defect (Sherrard 19[illegible] rosettes formed after acute trauma have been described by others in [illegible] al 1978) and *in vitro* (Sperling 1978) A characteristic feature for these [illegible] that the centre forms a point at which more than three cells meet depend[illegible] number of cells surrounding the cell which has been destroyed. The [illegible] complex of five cells described in Fig 3 is in fact a rosette The abnormal [illegible] complexes of four cells which was of far more frequent occurrence [illegible] not identical to such rosettes

According to Sherrard (1976) and Olson (1978) these rosettes became reorganized within a few hours leaving a cell pattern almost indistinguishable from a normal endothelium Based on *in vitro* studies Sperling (1978) noted that joint meetings of four cells may stay longer than the rosettes and suggested that joints of four cells actually may be formed from the rosettes The abnormal cellular intersections found in this study i e junctional complexes in which more than three cells meet may thus represent recent cell death

It was not possible to make valid estimation of endothelial cell loss due to large variation in cell size from one part of the graft to another and the inability to photograph exactly the same area after periods of time It may be suggested that a great cell loss occurred during the rejection episode (compare Fig 2 with Fig 5) and that the greatest cell loss occurred in the lower part of the graft as judged from the greater morphological changes and the greater thickness of graft in this region. Three months after onset (Fig 5) however no marked difference in cell density was observed between upper and lower part of graft. This indicates that a certain sliding of cells towards regions of lower cell density has occurred a phenomenon which has also been described after cataract extraction (Rao et al 1978)

The functional impairment of the endothelium accompanying the observed morphological changes was manifested through the changes in graft thickness (Fig 1) The return of a clear thin graft 2–3 weeks after onset of rejection was accompanied by the specular microscope appearance of a planar endothelium with no major intracellular or intercellular abnormalities although a certain degree of cellular pleomorphism still existed (Fig 4) This pleomorphism had reduced on the last examination (compare Figs 4 and 5) This indicates the process of active cell death and the immediate healing response is more deleterious for the barrier function of the endothelium than the process of reorganizing the cellular pattern from a state of pleomorphism to a more regular pattern

In this study non-contact specular microscopy has proved very useful in studying morphologic alterations of endothelial cells following an insult With the non contact technique the whole endothelium can be scanned without traumatizing the epithelium and interfering with subsequent specular microscopic examinations

Acknowledgments

The technical assistance of Mrs Anette Poulsen is gratefully acknowledged This work was supported by Fonden til Lægevidenskabens Fremme

References

Able M F Lee J & Rosenau W (1970) Lymphocyte-target cell i[illegible] *Path 60* 421–427

David J R (1973) Lymphocyte mediators and cellular hypersensitivity [illegible] 143–149

Ehlers N & Sperling S (1977) A technical improvement of the Har[illegible] *phthal (Kbh) 56* 53–66

Ehlers N & Bramsen T (1978) Management of late corneal graft [illegible] *(Kbh) 56* 984–997

Leibowitz H M Laing R A & Sandstrom M (1974) The effect of [illegible] chamber *Arch Ophthal (Chicago) 92* 227–230

Olsen T (1979) Optical principles for estimation of endothelial cell de[illegible] non-contact specular microscope *Acta Ophthal (Kbh) 57* 860–867

Olson L E Marshall J Rice N S C & Andrews R (1978) The effects of [illegible] corneal endothelium I The acute lesion *Brit J Ophthal 62* 134–144

Olson L E Marshall J Rice N S C & Andrews R (1978) Effects of [illegible] corneal endothelium II The endothelial repair process *Brit J Ophthal 62* [illegible]

Rao G N Shaw L E & Aquavella J V (1976) Morphological appearance of [illegible] corneal endothelium *Arch Ophthal (Chicago) 96* 2027–2030

Sherrard E S (1976) The corneal endothelium in vivo its response to mild trauma [illegible] *Res 22* 347–357

Sherrard E S (1978) Characterization of changes observed in the corneal endo[illegible] the specular microscope *Invest Ophthal Vis Sci 17* 399–3[illegible]

Sperling S (1978) Early morphological changes in organ cultured human [illegible] thelium *Acta Ophthal (Kbh) 56* 785–792

Author's address

Thomas Olsen Department of Ophthalmology
Århus Kommunehospital DK 8000 Århus C Denmark

Department of Ophthalmology (Head \ Ehle)
Århus Kommuneho pital
Uni ersity of Aarhus Denmark

TOXICITY OF DIMETHYLSULFOXIDE (DM°O) TO HUMAN CORNEAL ENDOTHELIUM IN ‹ T⁊O

BY

STEFFEN SPERLING and INGER G

Human cadaver corneas were obtained 10–1? h [illegible] leath incubated in organ culture for 20–28 h and exposed to [illegible] nd °% DMSO successively for 10 min at each concentration [illegible] cell damage was recognized by morphological alterations durin [illegible] f a coherent cell sheat after expulsion of damaged cells DMS [illegible] d to cause latent cell damage at 31 C No cell damage was found at 4 [illegible] n DMSO was dissolved in minimum essential medium (Eagle) plus [illegible] when DMSO was dissolved in human albumin plus sucrose DMSO found to be toxic at 4 C in pure serum and in Dulbecco s medium

Key words cryopreservation – DMSO toxicit – [illegible] lothel um – human cornea – latent injury – morphology – organ cultu e

[illegible]methylsulfoxide (DMSO) is the substance which so far has been most effective in [p]rotecting human corneal endothelium during deep freezing O Neill et al (1967) [f]ound by a technique of combined incubation and enzyme staining that canine and [h]uman endothelium was damaged in proportion to temperature concentration of [D]MSO and time of exposure Kaufman & Capella (1968) found by enzyme staining [t]hat 7 5% DMSO plus 10% sucrose in 25% human albumin was toxic to human [c]orneal endothelium above 10 C and atoxic below this temperature Schultz (1971) [a]nd Kanai et al (1972) found only slight ultrastructural changes in human [e]ndothelium after exposure to DMSO at 4 C in the media advocated by Kaufman & Capella (1968)

This study was undertaken after development of a sensitive method for detection of cell loss in human endothelium (Sperling 1978) The relation between DMSO toxicity temperature of exposure and basic medium was evaluated

Received February 13 1979

Material and Methods

Human eyes were enucleated 10–42 h post mortem. Cadavers were stored [illegible] and at 10 C during the remaining post mortem time. Eyes with [illegible] were rejected. In order to avoid bending of the corneas during enucleation [illegible] air was slowly injected in the vitreous until the ocular tension was [illegible] palpation. The eyes were rinsed in tap water. Corneas were excised with a 15 [illegible] sclera and stained by 0.3% trypane blue in 0.9% NaCl for one min. The [illegible] filled by 0.9% NaCl. The number of blue stained nuclei was counted in 10 [illegible] equivalating 0.13 mm² on cornea after random setting of the micros[illegible] flattened 6 mm of the cornea. The intercellular borders swell in NaCl. Four [illegible] the endothelial borders were obtained and three were selected for [illegible] photograph the cells in one test area equivalating 0.03 mm² on cornea were [illegible] methods of counting and selection of photographs and test areas described by [illegible] Gundersen (1978) were applied.

In 64 corneas from patients aged 13–91 years less than one per cent [illegible] endothelial cells were found. Patient age and time between death and incubation [illegible] in Fig. 1. These corneas were organ cultured for 20–98 h before use in the experiments. [illegible] technique described by Sperling (1979) was applied. Organ culture at 31°C in [illegible] minimum essential medium with Earle's salts, L-glutamin, $NaHCO_3$, eight per cent [illegible] and ten per cent fetal calf serum. In the following this medium is denoted MEM.

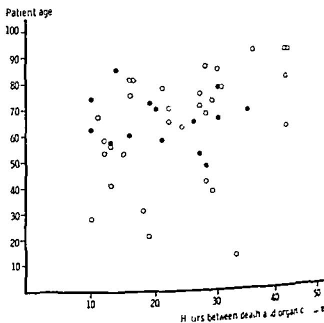


Fig. 1

Patient age and time between death and organ culture indicated for each of the 64 [illegible] corneas included in this study. An open circle represents one cornea. A closed circle represents two corneas from the same patient.

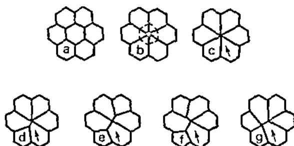


Fig 2

a-c Formation of a joint meeting of six originally hexagonal cells after loss of a central seventh cell
d g Transformation to joint meetings of four cells
Arrows indicate *reformation figure centres*

all experiments with DMSO corneas were subjected to 9 4 6 8 6 4 and 9% DMSO essively for 10 min in each concentration The toxicity of DMSO from Merck (Fur troskopie Uvasol 2950) and from Pierce (Silylation grade) was evaluated DMSO ity at 4 C was evaluated in MEM in fetal calf serum in Dubecco s phosphate buffered e with 5% serum (Dulbecco & Vogt 1954) and in salt poor human albumin (90% rma Rhodia 25% Cutter)

ccent cell loss was recognized as described by Sperling (1979) Staining of the endothelial borders in alizarin red and counting of reformation figures (cell constellation occurring ing reformation of the endothelial pattern after cell loss) Reformation figures are shown mnple in Fig 2b–g From each cornea four photographs were obtained and three were cted for counts On each photograph reformation figure centres (Fig) were counted in e frames each equivalating 0 076 mm² on cornea and the cells were counted in one frame valating 0 03 mm² Damage after exposure to DMSO was expressed as reformation ure centres (Fig 2) in per cent of cells per area

Results

ne between exposure and damage

enty corneas were exposed to DMSO (Pierce) in MEM at 31 C Four of these neas were evaluated immediately after the exposure and 16 were evaluated er 1 2, 4 and 24 h of organ culture at 31 C Four control corneas were organ tured for 24 h without exposure to DMSO

e percentages of reformation figures are indicated in Fig 3 The largest bers of reformation figures occurred one and two hours after exposure to MSO Very few reformation figures were found immediately after and four hours ter the exposure After 24 h of organ culture more reformation figures occurred the corneas subjected to DMSO than in the controls

mucosal epithelium in tissue culture (Jepsen 1978). DMSO (Merck) was used as in earlier experiments on bovine endothelium (Sperling 1976). Serum and albumin were originally used as basic cryoprotective media by Mueller & Smith (1963) and by Kaufman & Capella (1968). Dulbecco's medium was chosen because of the survival of bovine embryos stored and frozen in this medium (Trounson et al. 1976).

In the present study DMSO at 31 C caused an increased endothelial death 2 and 24 h after the exposure while this was not the case immediately after exposure. Latent damage was also found in human and canine corneas by O'Neill et al. (1967) and by Sperling (1976) in bovine endothelium. The delay between exposure to DMSO and the occurrence of cell death suggests that DMSO interferes with the cellular metabolism. The results of this study suggest two different sites of interference. One leading to irreversible cell injury within a few hours after the exposure and the other leading to a lowered cell survival in organ culture more than 4 h after the exposure to DMSO.

In this study DMSO was found to be toxic at 31 C and above at 4 C when dissolved in MEM. Increasing toxicity of DMSO with temperature was found in canine and human endothelium by O'Neill et al. (1967). In an earlier study on bovine corneas (Sperling 1976) increasing toxicity of DMSO with temperature was not found.

In the present study very slight cell damage was found when corneas were exposed to DMSO at 4 C in MEM or in albumin plus sucrose. Lack of DMSO toxicity to human endothelium at 4°C in albumin plus sucrose was found by ... (1971) and by Kanai et al. (1972). Parallel observations on rabbit endothelium have been reported by Kaufman & Capella (1968), van Horn et al. (19.. 1973), ... et al. (1973), Geroski & Edelhauser (1974) and by Basta et al. (1975). Lack of toxicity to human endothelium at 2°C in a medium comparable to MEM was found by Mueller (1968).

In the present study some cell damage was found in pure serum at 4 C but not in Dulbecco's medium. Damage occurred when DMSO was added to either of these media. Varied DMSO toxicity in different basic media was also found in bovine corneas (Sperling 1974, 1976). An explanation for this phenomenon may be that the toxicity of DMSO only becomes manifest in a suboptimal basic medium. This would parallel earlier observations of additional effects of unrelated ... trauma (Sperling 1974, 1976).

Conclusions

The toxicity of DMSO increases with temperature. For several quantities of ... the toxicity is very low at 4 C when DMSO is dissolved in basic media ...

...un or Dextran DMSO excerts latent damage to corneal endothelium This ...s that DMSO toxicity should not be evaluated immediately after the expose to ...O

Acknowledgments

...uthors wish to express their gratitude to Arne Jepsen Institute of Oral Pathology ... Dental Co ege Århus for inspiring discussions This study was supported by a grant ... the Danish Committee for Prevention of Blindness Laboratory technician Mrs B ...n, Department of Ophthalmology Århus Kommunehospital

References

...yd J F (1964) *Immunological methods* p 212 A symposium organized by the Council for ...ernational Organization of Medical Sciences etc Blackwell Scientific Publication ...ford.

... L Bourne W M & Blodi F C (1975) *Effect of various stages of cryopreservation on ...viability of corneal endothelium* *Ophthal Res* 7 133–139

...lla J A Kaufman H E & Robbins J F (1965) Preservation of viable corneal tissue ...h. *Ophthal (Chicago)* 74 669–673

...ecco R & Vogt M (1954) Plaque formation and isolation of pure lines with poliomyelitus ...uses *J exp Med* 99 167–182

...sb D H & Edelhauser H F (1974) *Metabolic evaluation of cryopreserved corneal ...ue. Arch. Ophthal (Chicago)* 91 130–133

...n A. (1978) Institute of Oral Pathology Royal Dental College Århus Unpublished ...*periments on DMSO in tissue cultures*

...n A Valenti J G & Kaufman H E (1973) Ultrastructural studies of cryopreserved ...rneal endothelium In Capella J A Edelhauser H F & van Horn D L eds *Corneal ...rvation* pp 248–269 Charles C Thomas Springfield

...man H E & Capella J A (1968) Preserved corneal tissue for transplantation *J ...urg* 1 125–129

...arey B E. Edelhauser H F & van Horn D L (1973) The effect of cryoprotection and ...odamage on corneal rehydration and endothelial structure *Cryobiol* 10 298–307

...ller F O (1968) Experiments on canine corneal donor material stored in liquid nitrogen ...*J Ophthal* 52 649–658

...ller F O & Smith A U (1963) Some experiments on grafting frozen corneal tissue in ...bits. *Exp Eye Res* 2 237–246

...ell P Mueller F O & Trevor Roper P D (1967) On the preservation of corneae at ...196 C for full thickness homografts in man and dog *Brit J Ophthal* 51 13–30

...ultz R O (1971) Laboratory evaluation of cryopreserved corneal tissue *Trans Ame ...thal. Soc* 69 563–608

...ng S (1974) Corneal cryopreservation e aluated by trypane blue staining *Ophthal R* ...23–35

...ahn S (1976) Toxicity of DMSO to bovine corneal endothelium *Ophthal Re* 8 ...35–40

...ng S (1978) Early morphological changes in organ cultured human corneal endo...thelium *Acta Ophthal (Kbh.)* 56 785–799

Sperling S & Gundersen H J C (1978) The precision of unbiased human ... density of endothelial cells in donor corneas. *Acta ophthal (Kbh)* 56 ...
Sperling S (1979) Human corneal endothelium in organ culture The ... rature and medium of incubation *Acta ophthal (Kbh)* 57 269-...6
Trounson A O Willadsen S M Rowson L E A & Newcomb R (1...) The ... eggs at room temperature and at low temperatures *J Reprod Fert* 46 1...
van Horn D L Edelhauser H F Gallun A B & Schultz R O (19...) ... ultrastructural freeze thaw induced injury *Arch Ophthal (Chicago)* ...
van Horn D L Edelhauser H F & DeBruin J (1973) Functional and ultras... in cryopreserved corneas *Arch Ophthal (Chicago)* 90 312-318

Author's address
Steffen Sperling Department of Ophthalmology
Århus Kommunehospital DK 8000 Århus C Denmark

Department of Ophthalmology (Head Thore Lie Thomassen)
Department of Clinical Neurophysiology (Head Arne J. S. Lundervold) and
Department of Neurology (Former head Sigvald Refsum)
Rikshospitalet, The National Hospital, University of Oslo

REFSUM's DISEASE

Eye manifestations in a patient treated with low phytol low phytanic acid diet

BY

EGILL HANSEN, NILS I. BACHEN
and TOR FLAGE

The cardinal eye symptoms of Refsum's disease are night blindness, retinal pigmentary degeneration and constriction of the visual fields. Similarities with or differences from retinitis pigmentosa are discussed. A 39-year-old male has had manifestations of Refsum's disease from the age of 7 years and has been on a low phytol low phytanic acid diet for the last 13 years. Peripheral ring scotomas were present. Some reduction of the visual fields has been recorded, but only in the far periphery. The central field has not been significantly constricted during a 7 year period. Very good visual functions were found within this area. All cone mechanisms were functioning at a normal level. A moderately reduced sensitivity level of the rod mechanism could be explained in a great measure by poor pupillary dilation in the dark. In this patient minimal or no progression of the visual findings was apparent. Probably an effect of treatment, there is little resemblance with ordinary retinitis pigmentosa. ERG showed moderate abnormality. Normal conducting time was found by VER. With fluorescein angiography a central area of normal appearance was sharply outlined in contrast to marked degenerations in paracentral regions.

Key words: retinal pigmentary degeneration – visual fields – colour vision – cone mechanisms – ERG – VER – fluorescein angiography – dietary treatment

Received February 9, 1979

In reports of Refsum s disease eye manifestations are generally described as [illegible] tary retinal degeneration of the retinitis pigmentosa type with night blindness [illegible] characteristic and early symptom A closer evaluation of the [illegible] selective registration of their response functions may be of particular [illegible] order to see in which way Refsum s disease accords with or differs [illegible] pigmentosa type of retinal dystrophy

Accumulation of phytanic acid in the serum is a characteristic of Refsum [illegible] and is probably the cause of some important neurological disorders Phytanic [illegible] not an endogenous product but is supplied with the food mainly as phytanic [illegible] as its precursor phytol As a consequence treatment with a low phytol [illegible] acid diet has been started (Eldjarn et al 1966) whereby regression [illegible] symptoms has been recorded though not visual and hearing loss [illegible] Refsum 1977) We report here the findings in a patient with Refsum s disease [illegible] a 13 year long period of dietary treatment

The patient

K M a male 39 years of age was seen at the hospital for the first time at the age [illegible] 7 years Six months previously slight paresis had developed in the lower [illegible] Subsequently ataxia ichthyosis and a slight hearing defect were also registered [illegible] the first ophthalmological examination a convergent squint of the left eye [illegible] moderate astigmatism and amblyopia was found Normal pupillary reactions [illegible] noticed There was nothing to remark from ophthalmoscopy One year later [illegible] and pepper pigmentation was noticed in the central part of the fundus [illegible] diagnosis heredopathia atactica polyneuritiformis" was made by Professor Refsum [illegible] when the patient was 8 years of age

Course The pigmentation of the fundus became more distinct at the [illegible] controls At the age of 27 years numerous pigment spots of the [illegible] type were found in a midperipheric zone of the fundus By perimetry [illegible] ring scotoma was recorded on the right side and an incomplete ring scotoma [illegible] left side A special diet low in phytanic acid was instituted from 1965 at the age [illegible] years A clear therapeutic effect was observed Reduced night vision was [illegible] noticed at the age of 8 years The patient admits that his night vision [illegible] only to a moderate degree as he has never had problems finding [illegible] His experience with colours has always been good The visual acuity [illegible] h is been normal (6/6)

Methods

[D]ark adaptation curves have been recorded with the Goldmann Weeker's adaptometer. [Peri]metric examinations under varying adapting conditions were performed with a modified [Go]ldmann perimeter (Hansen 1974, Hansen & Seim 1978). Near monochromatic inter[fer]ence filters (half band width 10–15 nm) were used. The angular size of the target is [ex]pressed in degrees and minutes. As the test target is slightly eleptic the diameter of the [eq]uivalent circular target comprising the same area is indicated. Background illumination [wa]s measured with a luxmeter (Hartmann & Brown EBLX3) in the bottom of the bowl. The [lu]minance of the test target is calculated as reflected energy at the level of the [s]phere.

Colour vision was examined with pseudo-isochromatic chart series, the Ishihara charts [(..th ed]ition) and the AO-HRR charts. Besides the Farnsworth's tritan chart, the Farns[wo]rth's D 15 test and the Farnsworth's 100-Hue test were used. Induced colour contrasts [we]re recorded with a series of tissue paper contrast charts (Hansen 1976). Anomaloscope [ex]aminations were performed with a Nagel anomaloscope type I.

Electro-retinographic examination was done by using a Lovac ERG contact lens (Medical [W]orkshop) with a refractive power of +100 D having a corneal electrode and a conjunctival [ref]erence electrode. By means of a signal averager the single flash-evoked ERG response was [rec]orded as the average of 50 single flash-evoked responses. The photostimulator was a Grass [PS2]2 stroboscope with 10 µs long electronic flashes. The intensity of the flashes was set at 8 for [the] photopic recording and at 1 for the scotopic.

Visual evoked response (VER). Flash train-evoked response patterns were recorded from [the] scalp with midline derivation O – C_z. Identical trains consisting of 40 flashes at 10 Hz were [pre]sented monocularly and the average response to 50 trains was recorded. By omitting one [fla]sh in the steady state part of the response pattern, it was possible to measure the latency [defined] as the time elapsed from the omitted flash to the cessation of the periodicity.

Dynamic tonometry. Pulse synchronous variations in intraocular pressure were registered [by] a method described by Hørven (1970).

A Nikon wide angle (45°) fundus camera was used for the fundus photography and the [flu]orescein angiography.

Ophthalmological findings

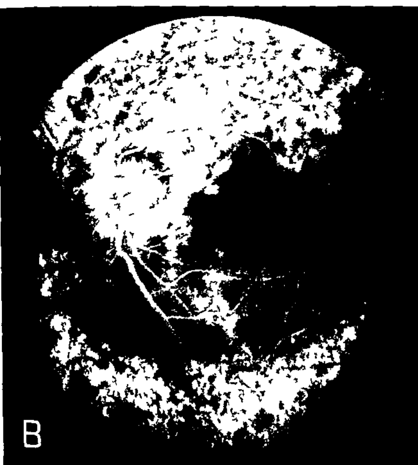
[Th]e patient had central and stable fixation with his right eye. There was unstable [fix]ation with the left eye, but no obvious strabismus. The objective findings in the [tw]o eyes were identical. There was no nystagmus. Strands of persistent pupillary [me]mbrants were present. The pupils were small, measuring 3 mm in diameter in [or]dinary illumination (100 lux). Direct and consensual reaction to light was normal. [The]re was slight reaction to accommodation. The pupils dilated poorly in darkness. [Aft]er [1]0 minutes in the dark the pupillary diameter was 4.5 mm (registered [pho]tographically). After repeated application of cyclogyl and metaoxedrine the [pu]pillary diameter measured 6 mm. Except for very delicate granular opacities in [th]e posterior cortex and stroma, the lenses were clear and so was the vitreous body. [Th]e optic discs were well marked, their colour within the normal variance.

Normally calibrated retinal vessels were seen in the *central fundus* The [illegible] vessels were moderately attenuated There was a peripapillary [illegible] Extended and marked degenerations were seen in the fundus near [illegible] including the upper part of the macula The *fovea* and lower part of the [illegible] a normal appearance (Fig 1 A) The degenerative changes indicated [illegible] the retina depigmentation of the pigment epithelium and loss of [illegible]



Fig 1

A Fundusphoto of the left eye showing peripapillary atrophic halo and [illegible] degenerative changes also affecting the upper part of the macula.
B Fluorescein angiogram in the late ven us phase left eye Hyperfluorescence [illegible] Marked degenerative changes as reflected by increased background fluorescence [illegible] fluorescence in the fovea as *normal*



vessels. The greater choroidal vessels had a normal appearance. Grey pigmentary areas and spots of pigments of bone corpuscular and ovular shape were seen towards the equator region. More peripherally, the fundus appearance changed from normal to atrophic areas.

Fluorescein angiography (Fig. 1 B) verified the finding of normally calibrated retinal vessels. The retinal circulatory phases were normal, and so was the choroidal flush. There was hypofluorescence of the optic disc suggesting some optic atrophy, a sign not revealed by the ophthalmoscopic examination. The degenerative changes were clearly outlined, especially those of the pigment epithelium, thus giving a sharp border between the degenerated and the normal fundus.

Visual acuity was 6/6 on the right eye and finger counting 1/2 m on the left eye.

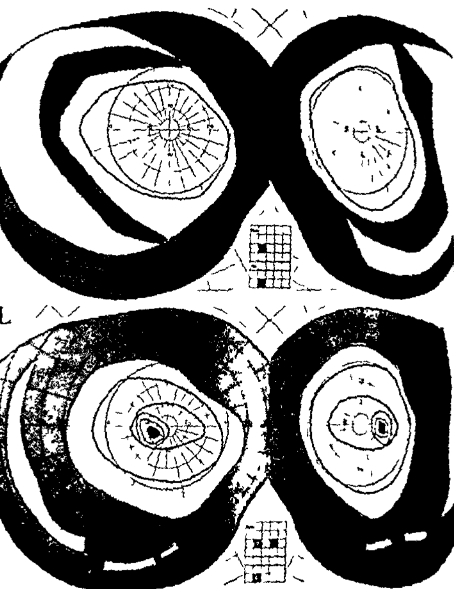


Fig 2

Registrations of the visual fields with a 7 years interval.
Recorded in *1971 (upper)* and 19 8 (lower)

Visual fields recorded in the Goldmann perimeter (*Fig 2*) show broad peripheral ring scotoma reaching the periphery on the nasal side *Compared* with the recording made in 1971 7 years previously there is a greater loss in the periphery leaving a smaller *crescent* of the field However the visual fields were not significantly changed in the central part

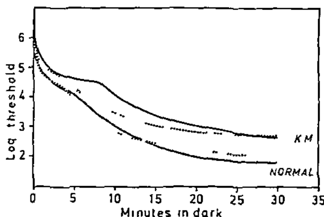


Fig 3

The dark adaptation curve of K M The normal mean values of the apparatus + 2 5 6 SD are shown. The dotted lines indicate the performance of a normal person with ordinary pupils (lower curve) and 2 h after instillation of pilocarpine (2%) in the eyes (upper curve) the pupillary diameter being 2½ mm

Special examinations

Colour vision

There was a normal performance with the Ishihara s test, the AO-H R R test the Farnsworth s D 15 test and the Farnsworth s tritan chart With the Farnsworth Munsell s 100-Hue test only a few chaps were confused (making 26 error scores) All the figures in a series of tissue paper contrast test charts could be seen With the Nagel anomaloscope he had precise settings within the normal range (41 5-43/16)

Dark adaptation

The course of dark adaptation has been repeatedly examined during the last 8 years showing essentially the same results There is a rather good response in the first phase and a moderate elevation of the thresholds during the second phase the final thresholds being about 1 log unit above the mean normal value (Fig 3) However the poor dilation of the patient s pupils in darkness (maximally 4½ mm) influences the result. The effect of miosis on the dark adaptation curve is shown by a normal observer Two curves were registered one with normal pupils and one with artificial miosis (2½ mm) caused by installation of pilocarpine (2%) in the eyes In the latter case elevated light thresholds were recorded the final thresholds being at the same level as those obtained for the patient

Quantitative perimetry

Static perimetry registered on the white standard background shows a high threshold sensitivity within the central area about 20 of eccentricity though with

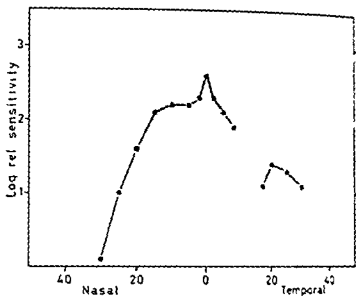


Fig 4
Static perimetry on standard white background (10 cd m²) right eye (Ø pt ver 3)
Shaded area indicates the normal variation (mean ± 2 SD)

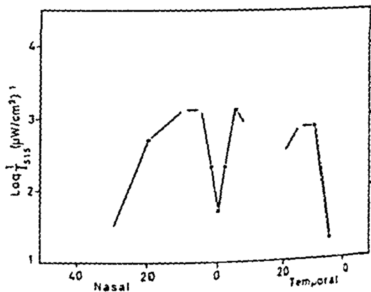


Fig 5
Static perimetry during total dark adaptation recorded with a green test (λ_{max} = 515 nm) subtending 27' angular diameter. Shaded area indicates variation (mean ± 2 SD) for 5 normals.

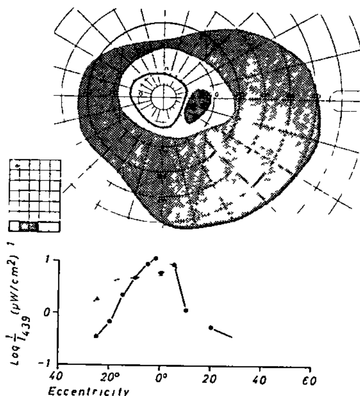


Fig 6

inetic and static perimetry against a yellow background (low pressure Na lamp λ=589 nm 00 lux) A blue target (λ_{max} = 439 nm) subtending 1 47 angular diameter is used The reatest isopter corresponds to the threshold sensitivity – 0 69 (μW/cm²)⁻¹ Dark area dicates the field loss in relation to a normal person of the same age of whom the static perimetry curve is also indicated (stippled line)

educed sensitivity near the blind spot (Fig 4) During total dark adaptation the atic perimetry curve is reduced by about 1 log unit in the central part of the field and more than 2 log units outside the 20 nasal and 30 temporal position (Fig 5) pectral sensitivity measurements against a yellow background show a good esponding blue mechanism with a peak sensitivity at about 439 nm A static erimetry curve obtained with the blue target (λ_{max} = 439 nm) demonstrates a high hreshold sensitivity of the blue cone mechanism in the central region within the 10 asal and 5 temporal position The extent of the visual field of the blue mechanism s more clearly shown by the kinetic perimetry record obtained with the same target gainst a yellow background (Fig 6)

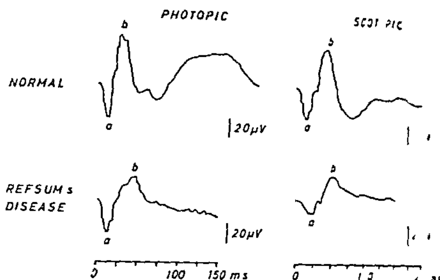


Fig 7

ERG records compared with those of a normal person under the same [illegible]

Electroretinography

The *photopic ERG* showed subnormal amplitudes the a wave [illegible] the b wave 22 µV (Fig 7) The peak time of the a wave 12 5 ms is normal [illegible] peak time of the b-wave 47 5 ms is significantly prolonged [illegible] value = 37 ms) Oscillatory potentials are present in the ascending [illegible] b-wave but with reduced amplitudes The *scotopic ERG* is also subnormal [illegible] amplitude of the a wave is 12 µV and that of the b-wave [illegible] µV The [illegible] take-off) of the a wave is 7 5 ms (prolonged) Prolonged peak time [illegible] the a and b-wave being 20 and [illegible]0 ms respectively

Visual evoked response

The average train-evoked response patterns from the right and left eye [illegible] tively showed well preserved periodicity in the steady state part The [illegible] recorded was 3[illegible] ms on each eye which is within the normal limits

Dynamic tonometry

The corneal indentation pulse amplitudes were moderately reduced [illegible] µ on each eye (mean normal value found by Hansen (19[illegible]) [illegible]

Other findings

lectromyography (EMG) and nerve conduction velocity

MG has been repeatedly recorded during the last 8 years of the observation eriod In 1970 and 1971 the EMG findings were markedly abnormal with very bundant denervation activity in all the muscle groups examined in the upper as ell as the lower extremities The occurrence of denervation activity has a ecreasing tendency Thus on the last examination in 1978 no denervation activity as found

In the first period after the start of the dietary treatment (in 1965) the conduction elocity of the peripheral nerves more than doubled It increased from 7 to 19 m/s n the ulnar nerve as reported by Refsum & Munthe Kaas (1966) and Stokke 1969) the normal value being about 50 m/s On later examinations 1968–1978 he values varied between 25 and 30 m/s

Neurological findings

A peripheral neuropathy was present showing atrophy of the muscles of the xtremities and incomplete loss of superficial and deep sensory qualities Besides here was anosmia and moderate neurogenic hearing loss of the cochlear type On omparison with previous neurological examinations it is obvious that the patient s ondition has improved The improvement was most clearly seen in muscular unction during the first two years after the start of dietary treatment concomitant with a fall in serum phytanic acid to a normal level While the muscle reflexes in the upper extremities prior to the dietary treatment were abolished they can now be licited

Discussion

The chief manifestations of heredopathia atactica polyneuritiformis are the eye symptoms and the neurological disorders comprising chronic polyneuropathy and ataxia In most cases cardiac disturbances and hearing loss also occur The disease is rare In a review by Refsum (1977) the total reported cases were 73 Unlike patients with retinitis pigmentosa patients do not generally live until old age

Since the introduction of the low phytanic acid diet, the prognostic situation has been considerably changed Being one of the first patients treated by Eldjarn et al (1966) our patient has now been on a low phytanic acid diet for 13 years The manifestations of the disease may therefore be much modified

The cardinal eye symptoms of Refsum's disease are night blindness, ... visual fields and a pigmentary retinopathy resembling retinitis pigmentosa. As in retinitis pigmentosa great significance has been ascribed to the ... However, in reports of Refsum's disease night blindness has been d... in a few cases by adaptometric registration. Edstrøm et al. (1959) f... photometric examination using the Cullstrand's method minimum pe... 30×10^{-5} lux in one patient, a definitely hemeralopic value. ... described adaptometric curves as "indisputably elevated", "certainly elevated", "slightly elevated". Night blindness is said also to be present in young patients at the beginning of the disease. In our patient night blindness had developed at the age of 8 years when the diagnosis was made. However, the night blindness described in this patient was of moderate degree, and was not pronounced at the age of 26 years when the dietary treatment was started. The dark adaptation ... recorded during the following years showed a moderate elevation of the ... thresholds. By evaluating the dark adaptation curves of this patient the ... dilation of the pupils in darkness should be taken into account. His ... thresholds are close to those found in a normal person with artificial miosis. ... Therefore, at the present stage our patient does not demonstrate any ... defect of night vision. As miosis and poor dilation of the pupils are ... findings in Refsum's disease (Refsum 1977), this should be allowed for in evaluation of the dark adaptation.

Pathological pigmentation in the fundus had developed during the first ... observation. Rapid development of pigment in Refsum's disease has been des... by Edstrøm et al. (1959) who initially found a bilateral retinal oedema ... protrusion, opacities in the vitreous bodies and precipitates in the anterior chamber of one eye. This was followed by retinal pigmentation one month later when the oedema had been absorbed. This suggests a chorioretinitic type of reaction at the initial stage of Refsum's disease which may possibly be induced by phytanic acid causing a toxic effect. Distinct homogenous flare in the anterior chamber and ... corpuscles in the vitreous was described in a child by Nordhagen & ... (1964) without, however, any other signs of uveitis.

Pigmentation in Refsum's disease most commonly appears as a fine ... pigmentation ("salt and pepper") while the "bone corpuscle" type characteristic of retinitis pigmentosa is more infrequent. As in retinitis pigmentosa, attenuated vessels and discoloured optic discs have been described as typical findings in advanced cases of Refsum's disease. This was not found in our patient except some slight attenuation of the retinal vessels in the periphery.

Fluorescein angiography demonstrates defects of the pigment epithelium ... atrophy around the optic disc and peripheral degeneration. Gekert & Be... (1969) in 2 patients found a normal passage of dye through the cho...

irculation a finding which is in accordance with the histopathological reports Alfandari (1971) in 3 patients found no features characteristic of Refsum's disease other than those seen in tapeto-retinal degenerations on examination with fluorescein angiography Dry et al (1976) in two new cases found diffuse changes in the pigment epithelium with peripheral pigmentary migration and important atrophic zones in the chorioretina

A moderate reduction of the corneal indentation pulse amplitudes was registered in our patient indicating reduced choroidal circulation Low pulse amplitudes are a typical finding in retinitis pigmentosa (Hørven 1970) In this case it may be consistent with an affected choroidal circulation in the periphery while a normal circulation may still be present in the central area Some optic atrophy could be traced by fluorescein angiography The normal latency found by VER however is indicative of a normal myelinisation of the optic nerve or only a slight degree of demyelinisation

As phytanic acid by *in vitro* experiments in peripheral nerves produces demyelinisation (Dubois Dalcq et al 1972) and demyelinisation actually has been found on autopsy (Goulon et al 1977) a prolonged latency time is to be expected in patients with Refsum's disease Optic atrophy with blindness has also been reported However no reports of VER examinations have been given from untreated nor from treated cases of Refsum's disease

There have been no reports of colour vision in patients with Refsum's disease except for one case reported by Alfandari (1971) having a very slight blue yellow defect as found by the 100 Hue test In retinitis pigmentosa blue yellow discrimination is most often affected (Verriest 1974) The finding of normal colour vision in our patient indicates a good cone function at a normal sensitivity level This was confirmed by the static perimetry in white light by registrating chiefly the long wavelength mechanisms A separate registration of blue cone function likewise demonstrates a normal level of sensitivity The typical finding in retinitis pigmentosa is a lack of rod response and a general depression of threshold sensitivity for all cone mechanisms but especially for the blue mechanism this being parallel to a tritan type of colour vision defect (Hansen 1977) Such changes may even be seen in early stages of retinitis pigmentosa

In most cases of Refsum's disease the ERG is extinguished Schott et al (1968) and Rougier (1970) describing the same patients found extinguished ERG in one patient and greatly reduced ERG (to 50%) in an affected brother 14 years of age A younger slightly affected brother had normal ERG In our case a subnormal ERG was found The increase in latency time of the a and b-waves is consistent with those found in dominantly transmitted pigmentary retinopathies (Babel et al 1977) where the oscillatory potentials are also preserved as in our case

The findings in our patient are only in part consistent with retinitis pigmentosa

Seen together with the complete loss of receptor functions in great per[illegible] there are remarkably good functioning receptors in the preserved central fi[illegible] feature not typical for pigmentary dystrophy Most probably the g[illegible] function in this patient is a consequence of the treatment with a low [illegible] diet. Both in retinitis pigmentosa and in Refsum s disease without trea[illegible] will be a progression of the visual disorder and a constriction of the visual fi[illegible] especially to be expected In our patient the progression has apparently [illegible] definite improvement in other respects has been observed as is shown by [illegible] conducting rate the EMC response as well as by the clinical improvement [illegible] and ataxia Only a few patients with Refsum s disease have been observed [illegible] treatment 2 patients of Eldjarn et al (1976) including our patient [illegible] Lundberg et al (1972) and 2 patients of Dry et al (1976) examined over 5 [illegible] spite of regression of neurological symptoms it is generally supposed that [illegible] failure and the hearing loss are not modified by the treatment In our p[illegible] has been no regression of the visual field defects and his hearing defect re[illegible] unaltered However approximately normal visual functions are preserved [illegible] central field No further constriction has occurred as could otherwise be exp[illegible] This certainly is a great therapeutic gain

Acknowledgment

We are indebted to Professor S Refsum for critical reading of the man[illegible] valuable suggestions

References

Alfandari N (1971) La maladie de Refsum Contribution a l'etude de ses m[illegible] oculaires These Universite Paris Ed medicales et universitaires Paris

Babel J Stangos N Korol S & Spiritus M (1977) Ocular elect[illegible] Thieme Stuttgart

Dubois Dalcq M Menu R & Bouse M (19[illegible]) Influence of fatty [illegible] cultured neurons J Neur path exp Neur [illegible] –667

Dry J Leynadier A Delporte M P & Lesvadier F (197[illegible]) A propos [illegible] maladie de Refsum Sem Hop Paris 5[illegible] –1681

Edstrom R Cr[illegible] O & Sandring H ([illegible]) Refsum s disease – three [illegible] Acta psychiat [illegible] 34 40–50

Eldjarn L Try K Stokke O Munthe Kaas A W Refsum S Steinberg D A [illegible] ([illegible]) Dietary effects on serum phytanic acid levels and on [illegible] hered opathia atactica polyneuritiformis [illegible]

Eldjarn L Stokke O & Try K (19[illegible]) Biochemical aspects of Refsum [illegible] for the dietary treatment In Vinken P J & Bruyn G W eds [illegible] Neurology Vol 27 Metabolic and deficiency diseases of the nerv[illegible] North Holland Publishing Co Amsterdam

Felzer A I & Berson E L (1969) Fluorescein angiography of hereditary retinal degenerations Arch Ophthal (Chicago) 81 7‾6–782
Goulon M., Gajdos Ph Chesneau A M & Raphael J-C (1977) Maladie de Refsum Thiebaut Klenk et Kahlke Ann Med interne (Paris) 128 869–876
Hansen E. (1974) The colour receptors studied by increment threshold measurements during chromatic adaptation in the Goldmann perimeter Acta ophthal (Kbh.) 52 490–500
Hansen E (1976) Examination of colour vision by use of induced contrast colours Acta ophthal (Kbh) 54 611–622
Hansen E (1977) Investigation of retinitis pigmentosa by use of specific quantitative perimetery Docum. ophthal (Den Haag) Proceedings ernes 14 461–472
Hansen E. & Seim T (1978) Calibration of the Goldmann perimeter and accessories used in specific quantitative perimetry Acta ophthal (Kbh) 56 241–251
Hansen I (1970) Dynamic tonometry Acta ophthal (Kbh) 48 23–38 and 39–58
Laudat P (1972) Intolerance au phytol Maladie de Refsum B ochim 54 735–740
Lundberg A Lilja L G Lundberg P O & Try K (1972) Heredopathia atactica polyneuritiformis (Refsum s disease) Experiences of dietary treatment and plasmapheresis Et p Neurol 8 309–324
Nordhagen E & Crøndahl J (1964) Heredopathia atactica polyneuritiformis (Refsum s disease) Acta ophthal (Kbh) 42 629–633
Refsum S Munthe Kaas A W (1966) Heredopathia atactica polyneuritiformis Nord Med 10 1320–1321
Refsum S (1977) Heredopathia atactica polyneuritiformis Phytanic acid storage disease (Refsum s disease) with particular reference to ophthalmological disturbances M tabol. ophthal 1 13– 9
Rougier J (1970) La maladie de Refsum Arch Ophthal (Paris) 30 665–672
Schott B Masson R. Quincy Cl Hosotte P & Da id M (1968) Étude clinique et biologique d une famille de maladie de Refsum Thiebaut Rev n u ol 118 230–236
Stokke O (1969) Dietary treatment of patients with Refsum s disease Effect of diet on serum phytanic acid levels In Try K. & Stokke O eds Biochem al and Dietary Stud in R f m D (Heredopathia Atactica Polyneuritiformis) Oslo Universitetsforlaget.
Vernest G (1974) Recent advances in the st ly of the acquired d f ie icie f c lour n Fondazione Giorgio Ronchi 24 Baccini & Chiappi Firenze

Author s address

Emil Hansen Ophthalmological Department
Rikshospitalet, The National Hospital Oslo 1 Norway

Department of Ophthalmology (Head: [illegible]), University of Helsinki, Helsinki, Finland.

CHARACTERISTICS OF OPTIC DISC IN HEALTHY SCHOOL CHILDREN

BY

H. ERKKILÄ and L. LAATIKAINEN

The ophthalmoscopic features of the optic discs were studied in a series of [illegible] non selected school children representing four age groups from [illegible] to 13 [illegible]. The distribution of the cup disc diameter ratios (C/D) showed that in the majority of the eyes (58.8%) the ratio was 0.[illegible]–0.3 independently of the age group. The highest ratio recorded, 0.7, was found in two eyes of the series. [illegible] asymmetry of 0.2 or more in the C/D ratios of the eyes was found in 5[illegible] of the children studied. The correlation between C/D ratio and the [illegible] of refraction was not statistically significant although C/D ratio of 0.4 or [illegible] was significantly commoner in myopia of −2.0 D or more than in the other eyes. A preponderance of large C/D ratio was also found in children with a [illegible] weight of 2500 g or less but the difference from the distribution in the [illegible] series was not significant.

Cilioretinal arteries were detected in 17.7% of the eyes and in [illegible] 3% of the subjects studied. In 8.1% of the children the condition was [illegible]. In children with a birth weight of 2500 g or less cilioretinal arteries were found in more than half of the cases.

Key words: optic disc – optic disc cup – cup disc ratio – cilioretinal artery – birth weight – refraction – epidemiology – child – school health

The normal optic disc shows great physiologic variation between one [illegible] and another. The role of inheritance in determining the cup disc diameter [illegible] ratio) was established by Armaly (1967). Later it was shown that the cup [illegible] varies with the size of the eyeball and the size of the optic disc (T[illegible] & [illegible]

Received February 22, 197[illegible]

969 Bengtsson 1976 Fledelius 1978) This lead to doubts regarding the value of the wide spread use of C/D ratio in the follow up patients with ocular hypertension

In newborn infants large cups and asymmetry in C/D ratio are uncommon Khodadoust et al 1968 Richardson 1968) In late childhood considerable changes occur in refraction (Sorsby et al 1957 Laatikainen & Erkkila 1979) and it has been estimated that after the age of 6 years the eye can still grow 0 2–0 3 mm a year up to or even after the age of puberty (Sorsby et al 1957) Thus changes in the optic discs might also be expected in late childhood Therefore in connection with a general ophthalmological survey of healthy school children presented in a separate paper (Laatikainen & Erkkila 1979) it was decided to study the C/D ratio and other features of the optic disc in children

Subjects and Methods

A non selected group of 411 comprehensive school children comprising 23 whole classes and representing four age groups from 7 to 15 years were examined The age distribution of the pupils is shown in Table I

The children underwent a thorough clinical ophthalmological examination Refraction was determined after cycloplegia (40–60 min after instillation of 1% cyclopentolate twice in each eye) C/D ratio was estimated using direct ophthalmoscopy by comparing the horizontal diameter of the cup to that of the optic disc The existence of optic disc drusen myopic conus and vascular or other abnormalities as well as the number and size of cilioretinal arteries were recorded

Results

C/D ratio

C/D ratio was analyzed in 814 eyes (in eight eyes this data had not been recorded) The cup was absent or minimal (C/D = 0 0–0 1) in 191 eyes (23 5%) In the majority 479 eyes (58 8%) the ratio was 0 2–0 3 and in 144 eyes (17 7%) C/D was

Table I
Age and number of subjects examined

Age (years)	Number
7 – 8	81
9 – 10	109
11 – 12	111
14 – 15	110
Total number	411

Table II

C/D ratio in the various age groups

C/D ratio	Age group								Total	
	7–8		9–10		11–1[illegible]		14–15			
	No	%	No	%	No	%	No	%	No	[illegible]
0 0–0 1	27	16 7	50	22 9	61	28.[illegible]	53	[illegible]	[illegible]	[illegible]
0 2–0 3	112	69 1	124	56 9	117	54 7	[illegible]	[illegible]	479	[illegible]
0 4–0 6	23	14 2	44	20 2	34	15 9	41	18 [illegible]	[illegible]	[illegible]
≈ 0 7	–	–	–	–	2	0 9	–	–	[illegible]	[illegible]
Total	162	100 0	218	100 0	214	100 0	[illegible] 0	100 0	[illegible]	[illegible]

0 4 or more in two of them 0 7 The C/D ratios showed no statistical differe[illegible] between the various age groups (Table II)

In most cases C/D ratio was symmetrical in both eyes In 19 of 40[illegible] cases ([illegible] the difference between the eyes was 0 2 and in 2 cases (0 5%) more than 0.2

Large cups (C/D ≧ 0 4) were proportionally more common in myopic than [illegible] other eyes (Table III) but the correlation between C/D ratio and refraction was [illegible] statistically significant ($\chi^2 = 5\ 307$ $0\ 2 < P < 0\ 3$) In eyes with myopia of [illegible] D or more C/D ratio of 0 4 was found in 9 of 24 eyes ([illegible]%) which differs significantly from the other eyes ($\chi^2 = 6\ 664$ $0\ 00[illegible] < P < 0\ 01$) The correlation [illegible]

Table III

Correlation of C/D ratio and refraction of the eyes

C/D ratio	Refraction (dioptres)					
	≧ + 2 0		+ 1 [illegible]5 – –0 2[illegible]		≧ –0 5 (≧ [illegible])	
	No	%	No	%	No	%
0 0–0 1	20	22 7	1[illegible]2	23 5	19 (5)	[illegible]
0 2–0 3	51	[illegible]7 6	388	60 1	40 (10)	50 0 [illegible]
≧ 0 4	17	19 3	106	16 4	[illegible]1 ([illegible])	[illegible]
Total	88	100 0	[illegible]16	100 0	80 ([illegible])	100 0 [illegible]

Table IV
Presence and size (large medium small) of cilioretinal arteries

Cilioretinal artery	Eyes with cilioretinal arteries	
	No	%
Large	21	2 8
Medium	35	4 7
Small	75	10 1
Total	131	17 7

large cupping and myopic conus was highly significant of the 19 eyes where conus was established C/D ratio of 0 4 or more was found in 11 ($\chi^2 = 21\ 596$ $P < 0\ 0005$)

Findings in eighteen children with a birth weight of 2500 g or less were analyzed separately In 10 of the 36 eyes (27 8%) C/D was 0 4 or more two of these were myopic (≧ −0 5 D) and four were hyperopic (≧ +2 0 D) The prevalence of large cups in children with low birth weight did not differ significantly from that in the whole group ($\chi^2 = 2\ 631$ $0\ 1 < P < 0\ 2$)

Disc vessels and cilioretinal arteries

An aberrant vascular pattern such as early branching of the retinal vessels on the optic disc was seen in six eyes

Cilioretinal arteries were detected in 131 of 740 eyes studied (17 7%) (Table IV) (In the 74 eyes of the 37 first examined children this data had not been recorded) In 30 of the 370 children (8 1%) the cilioretinal arteries were bilateral and in 71 children (19 2%) unilateral Thus 101 of 370 children (27 3%) had cilioretinal arteries in one or both eyes

In 20 eyes (2 7%) one or two large cilioretinal arteries were found on the temporal side of the disc extending beyond the macular area and supplying at least half of the macula In one eye a large cilioretinal artery was present on the nasal side In another 35 eyes (4 7%) a medium sized cilioretinal artery extended up to the fovea and supplied some portion of the macular circulation In 75 eyes (10 1%) cilioretinal arteries were small and did not participate in the macular circulation In 14 of the 131 eyes more than one cilioretinal artery could be seen

The distribution of refractive errors or C/D ratios in eyes with cilioretinal arteries did not differ from that for the whole series (Tables II and V) Table V shows that large cilioretinal arteries were common in eyes with minimal or no cupping (9/31)

and on the contrary infrequent in eyes with a large cup size (1 9%) The [illegible] was however not statistically significant ($\chi^2 = 1312$ $0 1 < P < 0 9$)

Cilioretinal arteries were commoner in children with low birth weight than in the whole group one or two cilioretinal arteries were found in 8 of the 15 [illegible] studied (53 3%) ($\chi^2 = 5 696$ $0 01 < P < 0 05$) In 10 eyes the cilioretinal [illegible] were small in two eyes of medium size There was no correlation between C/D [illegible] and the presence of cilioretinal arteries in children with low birth weights

Other findings on the optic discs

Optic disc drusen were diagnosed in three subjects in one of them bilaterally [illegible] child had bilateral cilioretinal arteries as well One of the other two had a cilioretinal artery in the contralateral eye In addition drusen was suspected in [illegible] eyes Myopic conus was present in 19 eyes and a tilted disc was found in four eyes

Discussion

According to Elschnig (1901) formation of the physiologic cup of the optic disc [illegible] modified by the extent of connective tissue during the phase of embryonic development in which the axons of the ganglionic cells approach the disc If the connective tissue has been completely reabsorbed the axons might fill the [illegible] scleral foramen completely and the disc would therefore have no real physiologic cup

In the present study minimal or no cupping was found in 23 5% of the children Similar percentages have earlier been presented on adults by Ford & Sarwar [illegible] 27 04% and by Hollows & McGuiness (1966) 23% The similarity of these figures [illegible]

Table 3
C/D ratio in eyes with cilioretinal arteries

C/D	Eyes with cilioretinal arteries			Total	
	Large	Medium	Small	No	%
0 0–0 1	9	8	14	31	[illegible]
0 2–0 3	11	20	48	[illegible]9	[illegible]
≧0 4	1	7	13	[illegible]1	
Total	21	35	75	131	[illegible]

Table VI
Survey of reported frequencies of cilioretinal arteries (CRA)

Author	No of subjects examined	% of eyes with CRA	% of subjects with CRA (unilateral/bilateral)
ackson (1911)	500	19 1	29 6 (21 0/ 8 6)
Collier (1957)	1000	12 5	21 6 (18 2/ 4 3)
orentzen (1970)	172	15 0	26 0 (?2 0/ 4 0)
odeno (1974)	956	39 6	
ustice & Lehmann (1976)	1000	32 1	49 5 (34 9/14 6)
Jouzas & Markakis (1978)	?000	26 3	42 6 (34 1/ 8 5)
Present series	370	17 7	27 3 (19 2/ 8 1)

ll as the finding that *there was no significant difference in C/D ratio between the* rious age groups in this series indicate that there is no general enlargement in the p size at school age or after except in eyes with moderate or high myopia In the e group of 7–8 years a C/D ratio of 0 4 or more was noted in 14% of the cases ich considerably exceeds the 2 77% frequency in infants presented by Richard n (1968) Thus it seems that some increase in cup size occurs during early ildhood

There is general agreement about the symmetry of the physiologic cupping tween the two eyes (Snydacker 1964 Hollows & McGuiness 1966) In newborn fants *marked asymmetry of disc cupping has been reported in 2 3% by Richard* on (1968) and in 3% by Khodadoust et al (1968) In this series a difference of 0 2 greater was fairly common in 5 2% of the cases which is between the cited gures in infants and those in adults presented by Armaly (8%) (1967)

Physiologic variation in refraction seems to have no significant effect on C/D tio Absent or minimal cups were equally common in myopic (23 8%) and peropic (22 7%) eyes A high C/D ratio (0 4 or more) was however significantly ore common in myopia of −2 0 D or more particularly if myopic conus was resent, reflecting an enlargement of the posterior scleral foramen in axial myopia

Pronounced *cupping of the optic disc has been shown in children with low birth* eight (Fledelius 1978) In the present study also the prevalence of large cups was eater in children with low (2500 g or less) birth weight than in the whole series ut the difference was not statistically significant In children with low birth weight lioretinal arteries were also common but their presence did not correlate with the ze of the cup

The prevalence of cilioretinal arteries reported in the literature varies from 13 to

40% of the eyes (Table IV) The great variation might be explained by the unsimilar methods used The highest figures of 39 6 and 3? ?% of the eyes were estimated by means of fluorescein angiography (Sodeno 19?4 Justice & Lehmann 1976) In the present series the frequency of cilioretinal arteries was estimated in 17 7% of the eyes approximating the figure of 15% defined ophthalmoscopically by Lorentzen (1970) in Denmark Bilateral cilioretinal arteries were found in ? of the subjects who had cilioretinal arteries which corresponds the estimation of Jackson (1911) and Justice & Lehmann (1976) Bouzas & Markakis (19?8) reported bilateral cilioretinal arteries in 20% and Lorentzen (1970) and Collier (1957) in less than 20%

Mann (1928) attributed the development of cilioretinal arteries to the enlargement of anastomoses of the posterior ciliary arteries with small branches from the hyaloid artery on the disc The higher frequency of cilioretinal arteries in infants with low birth weight support this concept Thus theoretically factors involved in the reabsorbtion of the hyaloid arterial system could affect both on the presence of cilioretinal arteries and the development of physiologic cupping In the present study we could not however find any correlation between the size of the cup and the presence of cilioretinal arteries

References

Armaly M F (1967) Genetic determination of cup disc ratio of the optic nerve Arch Ophthal (Chicago) 78 35–43

Bengtsson B (1976) The variation and covariation of cup and disc diameters Acta ophthal (Kbh) 54 804–818

Bouzas S & Markakis G (1978) Observations cliniques concernant les arteres cilio retiniennes In *5th Congr of Europ Soc Ophthal* Hamburg 19 ? pp 13?–13? Enke Verlag Stuttgart

Collier M (1957) Frequence des vaisseaux cilio retiniens leur rapport avec les arteres leur association avec d autre anomalies du fond de l oeil *Bull Soc Ophtal Fr* ? 598–601

Elschnig A (190) Der normale Sehnerveneintritt des menschlichen Auges Denkschr *Mat naturais Classe Wien 70* 219–303

Fledelius H (1978) Optic disc cupping and prematurity Large cups as a possible low birth weight sequel *Acta ophthal (Kbh) 56* 563–5?3

Ford M & Sarwar M (1963) Features of a clinically normal optic disc *Brit J Ophthal* ? 50–52

Hollows F C & McGuiness R (1966) The size of the optic cup *Trans Ophthal Soc ?* 33–38

Jackson F (1911) Cilio-retinal and other anomalous retinal vessels *Ophthal Rec* ?

Justice J Jr & Lehmann R P (1976) Consideration of the cilioretinal circulation *Ophthal Proc Ser No 9 (Den Haag)* 261

Khodadoust A A Ziai M & Biggs S L (1968) Optic disc in normal newborns *Amer J Ophthal 66* 502–504

Laatikainen L & Erkkila H (1979) Refraction errors and other ocular findings in school children *Acta ophthal (Kbh) 57* (in press)

Lorentzen S E (1970) Incidence of cilioretinal arteries *Acta ophthal (Kbh) 48* 518–524

Mann I (1928) *The development of the human eye* pp 233–234 University Press Cambridge

Richardson K T (1968) Optic cup symmetry in normal newborn infants *Invest Ophthal 7* 137–140

Snydacker D (1964) The normal optic disc *Amer J Ophthal 58* 958–964

Sodeno Y (1974) Cilioretinal artery and the microcirculation of the optic disc *Acta Soc ophthal jap 78* 561–575

Sorsby A Benjamin B Davey J B Sheridan M & Tanner J M (1957) *Emmetropia and its aberrations* Spec Rep Ser No 293 Med Res Council London

Tomlinson A & Phillips C I (1969) Ratio of optic cup to optic disc In relation to axial length of eyeball and refraction *Brit J Ophthal 53* 765–768

Authors address
H Erkkila and L Laatikainen
Department of Ophthalmology Helsinki University Central Hospital
Haartmaninkatu 4C SF-00290 Helsinki 29 Finland

Department of Ophthalmology and Department of P[illegible] St Joseph's Hospital Reykjavik Iceland

OCULAR TRAUMA

Observation in 105 patients

BY

ULFAR THORDARSON ARNI T RAGNARSSON
and BJÖRN GUDBRANDSSON

A report on 105 cases of ocular trauma hospitalized in St Joseph's Hospital in Reykjavik during a 12 year period is presented Of these patients 94 were males and 11 females Thirty nine patients were children and only six patients were older than 50 years Most comon were occupational accidents (45 cases) and of these 29 were connected with industry Other main causes were traffic accidents sport and dangerous play by children Of the total 105 cases 70 had perforation of the bulb All but nine underwent some kind of operation and enucleation was performed in 10 patients Nearly two thirds of the patients were hospitalized within six hours of the accident and 84% within 24 h.

Key words ocular trauma – causes of – age distribution of

The Department of Ophthalmology St Joseph's Hospital is the only ophthalmological clinic in Iceland and therefore serves all of the 230 000 inhabitants. Thus all cases with major ocular trauma occurring in the country are treated at the clinic.

This study aims at observing the causes of the ocular trauma, age and sex distribution of the patients and the kind of ocular trauma We have also [illegible] noted the outcome after treatment

Received November 15 1978

Material and Methods

We selected randomly 105 cases with *major ocular trauma treated at the St. Joseph's* Hospital Reykjavik during the period 1965-1976 These cases ought to roughly represent all cases of ocular trauma occurring in the country during this period The total number of patients treated at the clinic during this period was much larger The study was done retrospectively by examining the hospital records and by telephoning those who could be reached (50 patients) and asking for the vision of the damaged eye

Results

Age distribution

This is shown in Fig 1 children (≤15 years) comprised 39 patients (37%) which is about the same ratio as in other studies made on this subject Only six patients were older than 50 years

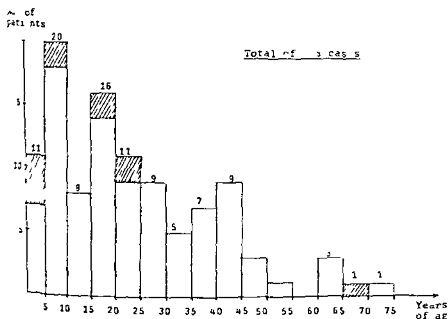


Fig 1

The age incidence of the 105 cases of ocular trauma discussed in the article Shaded areas stand for females Note the high incidence of children aged 15 years or less (39 cases) and that only six cases occurred after 50 years of age

Table I
The cause of ocular injuries in the 39 children discussed in the article

Cause of injury	Number of cases
Pointed objects (knives etc.)	6
Explosive materials (fireworks etc.)	4
Fall	4
Sport (winter sport 3 handball)	4
Arrows	3
Dart arrows	2
Catapulted missiles	2
Animals	2
Glass fragments	2
Miscellaneous	10
Total	39

Table II
The activities of the 103 patients discussed in the article at the time of injury

Occupational injuries			43
I Industry		29	
Construction and building	14		
Automobile repair	7		
Engineering and cutlery	6		
Other	2		
II Fishing		3	
III Agriculture		4	
IV Miscellaneous		[illegible]	
Road accidents			8
Sport injuries			3
Domestic injuries			10
Miscellaneous (such as playing of children etc)			29
Total			103

Sex distribution

In this study 94 (90%) were males and 11 (10%) females Eight of the females were younger than 20 years See Fig 1

Causes

When discussing causes of ocular trauma one can consider both the circumstances (place of the accidence or the activities of the victim) and the object causing the trauma. For obvious reasons it is usual to classify the causes according to the former See Table II

Occupational injuries 43 of the total 105 cases (40%) are in this group or 65% of the ocular trauma in adults As would be expected most of these occurred in industry or 29 cases which comprises 28% of the total and 44% of the adults in the study Perforation of the eye occurred in 17 of the 29 cases Most commonly it was due to ng wire on building sites (9) splints of metal or wood (8) screwdriver (6) and nails (9)

Industrial accidents are the most common cause of ocular trauma in Western Europe and ocular trauma represent 3–5% of all industrial accidents according to Duke Elder s text book. Because of the sparsity of heavy industry in Iceland most of the accidents happen in construction and building and in light industry

Only four cases could be traced to *agricultural work* three of which were engaged in repairing fences

Three cases could be traced to *fishing* accidents which is surprisingly low when taking into account the large number of fishermen in Iceland

Traffic accidents Eight cases with (which might be expected) severe trauma At least two patients became totally blind in the eye and four others had perforation of the bulb

Sport injuries Only five cases of which four were in children Two cases connected with skiing 1 skating 1 handball and 1 fishing According to this most eye injuries connected with sport are minor and therefore can be treated ambulatorily

Domestic injuries Twenty cases had ocular injuries in the home 10 children and 10 adults Of the adults four cases were connected with the use of alcohol (in the whole study 11 of 105 cases were connected with this) The most usual causes of injuries in the home were pointed objects glass fragments and falls

Miscellaneous This group comprised 29 cases where the largest sub-group was outdoor playing of children

Table III

The time interval between the time of ocular injury to the time of h[illegible] admission with regard to the geographical location of the accident. Man[illegible] the patients were seen by a doctor before the admission and partially treated by him

Location of accident	Time interval from accident to admission		
	≤ 6 h	≤ [illegible] h	Total
Stor Reykjavik and Reykjanesskagi	45 (75%)	52 (8[illegible]%)	[illegible]
Suðurland	4 (44%)	8 (88%)	9
Vesturland	7 (88%)	8 (100%)	8
Vestfirðir	2 (50%)	3 ([illegible]5%)	4
Norðurland	3 (25%)	8 (6[illegible])	1[illegible]
Austurland	3 ([illegible]7%)	8 ([illegible]3[illegible])	11
Total	64 (6[illegible]%)	87 (84%)	101

Children

Because of the large number of children in the study we want to deal with [illegible] group a little more closely (see Table I) The total number of children in the [illegible] 39 of which 33 were boys and 6 were girls Rather severe injuries are usual in [illegible] group with at least 6 cases who totally lost light perception in the injured eye [illegible] other (60%) had perforation of the eye Three had contusion of the eye ball [illegible] developed traumatic cataract Of the remainder most had a wound in [illegible] eyelid or the lacrimal duct

Time elapsing from accident to admission

This is of interest because the clinic serves the whole country which has a [illegible] difficult communication system Sixty four patients were hospitalized within [illegible] first six hours of the accident and 87 patients within 24 h Generally the [illegible] cause of delay in this group was the long distance from the clinic but in the group which were hospitalized after 24 h the most common cause was that the [illegible] the eye injury was not recognized by the patient (see Table III)

The nature of the eye injury

As evident in Table IV most of the injuries were serious otherwise they would had been treated ambulatorily Of the 105 cases 70 patients had perforation of the [illegible]

Table IV

The types of injury in the 105 cases of ocular trauma. Concomitant perforation of the cornea and prolapse of the iris as the only injuries was described in 25 cases and of these 6 cases developed traumatic cataract

Type of injury (diagnosis)	As only injury or concomitant with other eye injuries	As the only eye injury
Ruptura bulbi	10	10
Perforatio corneae	42	12
Prolapsus iridis	27	0
Cataracta traumatica	10	0
Vulnus conjunctivae	13	6
Vulnus palpebrae	17	4
Ruptura canaliculi lacrimalis	8	2
Corpus alienum bulbi	9	4
Vulnus sclerae	9	1
Contusio bulbi	5	2
Vulnus musculi ocularis	3	0
Corpus alienum corneae	3	2
Prolapsus corporis vitreosum	4	0
Expulsio lentis	3	0
Trauma orbitae	2	0
Prolapsus uveae	2	0
Prolapsus corpora ciliare	1	0
Iridocyclitis traumatica	1	0
Endophthalmitis	1	0
Ophthalmia sympathetica	1	0
Ablatio retinae	1	0

Treatment

The purpose of this paper is not to make a detailed discussion on the treatment of the ocular traumas. All but nine of the 105 patients had to undergo some kind of operation. Enucleation had to be performed in 10 patients. Most patients with perforation of the bulb were treated with antibiotics and cortico-steroids.

The vision on the affected eye after treatment

We tried to call all the patients by telephone and managed to reach 50 out of the 105. They were asked about the sight on the involved eye. The results are shown in Table V.

Table 1

The subjective valuation of sight by the 50 patients contacted for follow-up (regardless of the use of eyeglasses or lenses)

Total	Blind	Sees light	Sees outlines	Can read	Sees well in the distance and for driving	Perfect sight
50	6	4	5	1	[illegible]	32

Of these 50 patients 32 considered that they had perfect vision on the injured eye six patients had complete blindness of the eye and nine patients had very poor vision Of the group which we did not reach eight patients had undergone enucleation Therefore at least 14 patients of the 105 became blind in the injured eye Of these 14 patients six were children and most of the rest were under 30 years of age with mean age of 22 years

Discussion

The results speak for themselves and need no long discussion In general we can say that the causes of the ocular trauma in our study are much the same as in previous studies on the same topic The study stresses the need for prophylactic measures to prevent eye injuries both occupational (especially within industry) and eye injuries of children

The age distribution shows that ocular trauma occurs mainly in young people and as previously known it is the most common cause of unilateral blindness in children and young adults Females comprise only 10% of the total number of patients and people over 50 years of age are seldom involved

References

Bertoni & Zedda (1967) Ocular trauma in 653 children *Rass med* [illegible] p 451
Duke Elder S (1972) *System of Ophthalmology* vol XIV part I pp 3–48
Holland (1961) Ocular trauma in 215 children *Klin Mbl Augenheilk* 139 p [illegible]
Lambah (1962) Ocular trauma in 610 children *Lancet* [illegible] p 1351
Werner (195?) Ocular trauma in 215 children *Acta ophthal (Kbh)* 30 p 9[illegible]

Author's address

Ulfar Thordarson M D Department of Ophthalmology
St Joseph's Hospital Reykjavik Iceland

Department of F perimental Ophthalmology
L niversity Eye Clinic (Head C E T Krakau)
and the Dalby Commi nity Research Centre (Head Ake Norden) Lund S eden

AUTOMATIC PERIMETRY IN A POPULATION SURVEY

BY

BO BENGTSSON and C E T KRAKAU

Automatic perimetry was performed in 2998 eyes of 1511 subjects comprising 8" of all persons born 1907–1921 and resident in a certain small area Unreliable or abnormal tests were repeated The average number of tests per person was 2 25 About 90% of all tests in normal eyes were performed in less than 3 min The screening was considered negati e in 2887 eyes incomplete in 0 eyes and positive in 91 eyes (3%) Fighteen of the positives were pre iously unknown glaucomatous defects There was only one unexplained false positive We concluded that the method is quick sensitive specifi and dependable The apparatus is simple to manage and cheap to run

Key word defined population – visual field defects – automatic perimetry – glaucoma.

During the last few years an automatic perimeter has been developed and tested at the Department of Experimental Ophthalmology in Lund (Heijl & Krakau 1975a,b Krakau 1978) A great deal of clinical experience was gained i a. the instrument was used on a material of glaucomas and glaucoma suspects (Heijl 1976) A trial in a population survey of the logic for detection of glaucomatous visual field defects was considered appropriate

A preliminary report comprising about one third of the present material was given at the Nara Symposium (Bengtsson & Krakau 1979)

Received March 5 19 9

Material

All persons born 1907–1921 and resident in the district provided for by [illegible] Community Care Centre were listed in December 1976 The list was [illegible] according to the residential addresses and kept up to date by means of [illegible] reports from the County Council on removals and deaths Following [illegible] the inhabitants were contacted in rotation during 1977 and 1978 and [illegible] repeated ophthalmological examinations Attempts at persuasion were [illegible] only persons able and willing to attend within a few weeks were included [illegible] survey

One person known to be blind and 24 patients subjected to anti[illegible] therapy were not invited There were only five cases with field defects [illegible] congenital and four secondary glaucomas) in this group

Out of 1938 invited persons 1511 (78%) took part in the survey The [illegible] attendance was largely independent of age and sex

Six persons had lost one eye 16 were blind in one eye and 2 were unable [illegible] straight ahead with one eye Automatic perimetry was attempted however [illegible] one of the 2998 seeing and mobile eyes of the 1511 subjects in the survey

Methods

Sphygmomanometric measurement of the systemic blood pressure determination of the visual acuity subjective refraction automatic perimetry fundus photography indirect ophthalmoscopy slit lamp examination and Goldmann [illegible] were attempted in every case Perimetry and photography were conducted by two alternating assistants – ophthalmoscopy slit lamp examination and [illegible] one of the authors (BB) Perimetric data handling was entirely automatic and [illegible] data were immediately recorded on special forms Transfer to magnetic tape and further processing were performed at the Computer Centre in Lund.

In the present report we have confined ourselves to the results of [illegible] perimetry

Perimeter The perimeter described by Heijl & Krakau (1975) was used [illegible] points are fixed in a pattern of concentric circles at 5 10 and 15 degrees [illegible] tricity At 20 degrees there are eight points placed as shown in Fig 1 At each [illegible] point a LED (light emitting diode) can be made to emit light at any one of 16 [illegible] The relation of luminance between two consecutive levels is 1 2 One further [illegible] spot is projected on to the perimeter screen adjusted so as to fall in the blind spot area and lit at random intervals on an average 1/9 of all trials. If the patient [illegible]

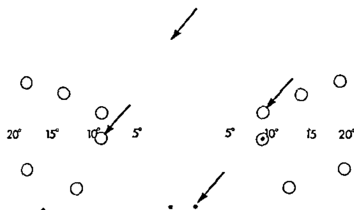


Fig 1

Location of test points The threshold is first determined at the four places pointed at by arr s When one or two of the points ( ) and eventually one or two of the points (◯) are n t seen the blind spot is denoted normal (The right group refers to the right e e and vice versa) The central point is a red fixation light

his fixation it cannot be seen The patient signals that he has perceived a light by pressing a button

Test l me The test procedure is similar to the programmes for glaucoma screening by manual perimetry as used by Armaly Drance and others However the points tested are chosen in random order in the computerized version Minor changes only have been made in the programme used by Heijl (1976) The *threshold* is at first determined at four points on the 10 degree circle (Fig 1) If at a certain point, a target is seen next time it will be shown on a one step fainter level and vice versa The target perceived is accepted as the threshold when the process has changed its direction three times A *supraliminal* test value is calculated from the thresholds of the first four points and used as a starting level for the remaining test points If at any point this level is seen this point is not further tested if not the intensity is increased by one step If this level is seen this point is again not tested if not seen the LED is lit at its very highest intensity level If still not seen it is not tested again If the highest intensity level is seen the process returns to the supraliminal starting level passes step by step to higher intensities as long as the light is not seen and ends as soon as the light is seen again (Fig 2)

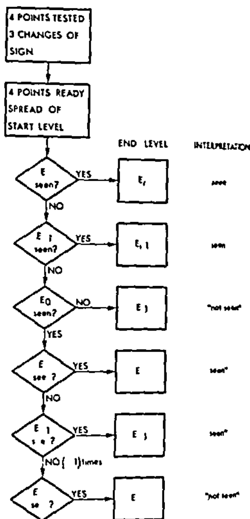


Fig 2
Flow diagram showing the course of testing one point F start level
F highest intensity level

Test conditions As a rule the blind spot stimuli for fixation control were directed towards a point about 2 cm temporally and inferior to the test diode 15 temporally to the fixation light An individual adjustment was resorted to only when an anomalous position of the blind spot was suspected Only spheric glasses were used to correct for near vision and no special steps were taken to secure dark adaptation The instructions to the subjects (to fixate the red light and to press the button whenever a white light was seen) were kept as few and simple as possible The assistant remained in the room for a while but left it as soon as she felt that cooperation was satisfactory

Presentation of results The results were presented by means of a keyboard printer (Silent 700 TEXAS Instruments) The printout of one field chart required less than 1 min

Interpretation. A test point was considered *seen* if it had ended on a level not more than one step lower than the initial one (Fig 2)

Up to four points not seen in the blind spot area (Fig 1) were accepted as a *normal blind spot*. If the blind spot was missed and in addition the blind spot check light seen more than five times the test was regarded as *unreliable*

A reliable test was considered *normal* if all points outside the normal blind spot were seen (otherwise *abnormal*) If a reliable test was normal the eye was classed as *negative*

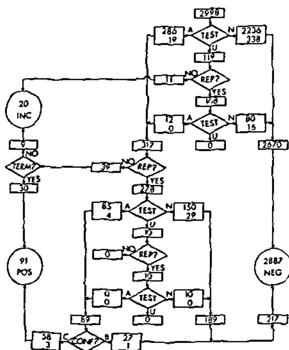


Fig 3

Flow diagram used when classifying the field screening of an eye as positive or negative The number of eyes following a certain course is given in the cartouches The upper number refers to tests in which the blind spot was found – the lower to tests in which it was missed.

REP?	= Repetition of test?	U =	Unreliable tests
TERM	= Termination of screenings?	N =	Normal tests
CONF	= Confirmation of defects	A =	Abnormal tests
INC	= Incomplete screenings	B =	Unconfirmed defects
NEG	= Negative screenings	C =	Confirmed defects
POS	= Positive screenings		

When a reliable abnormal test was obtained in an eye with a known field defect or in which a defect could be attributed to an ophthalmoscopic finding the screening was *terminated* and considered *positive* With this exception the screening of one eye was not considered *complete* unless all unreliable or abnormal tests were repeated

If the first reliable test was abnormal a *proposed defect* was assumed If such a defect was followed by a similar one in the same eye the points outside the area of the blind spot were compared If at least one of those points was not seen in both tests the second test showed a *confirmed defect* and the screening was considered positive If a proposed defect was not reproduced at any point in the second test the screening was considered *negative*

The final classification of screenings as "*incomplete*" *positive* or *negative* followed rules so strict that the computer was entrusted with it (Fig 3) The operator had to initiate a retest but even this task could have been left to the computer The ophthalmologist had to decide whether a termination of the screening was warranted (and to reclassify terminated screenings from incomplete to positive)

As indicated in the preceding paragraph even the immediate interpretation of each test ordering a retest according to the strategy adopted etc could have been left to the computer In the course of the survey a major part of the software necessary for a complete automation was developed for the purposes of [illegible] and arranging the material For a start however we had to resort to a semi-automatic procedure involving a presentation of the results in a cruder form and a human element – which was retained throughout the entire study

Results

The background illumination was in nine eyes with nuclear cataract 0 1 cd/m² and in others 1 cd/m² There was no difference in the threshold reached between the first (right) and the second (left) eye Thus there was no indication of [illegible] adaptation

The *time* needed for one test was less than 3 min in 90% of all normal eyes The average number of tests per person was 2 25

Eleven unreliable and 9 abnormal tests were not repeated – by inadvertence [illegible] subjects) or because the subject was not motivated to continue (9 subjects) but a field screening was completed in 2978 eyes – 2887 negative and 91 (3%) positive (Fig 3)

The positive field screenings are summarized in Table I

Six *known* (nonglaucomatous) *field defects* were all spotted by the [illegible] perimeter We also soon learned to expect [illegible] *defects or* [illegible] *spots* in highly myopic eyes with posterior staphyloma and or peripapillary atrophy

Table I
Positive screenings

	No of cases n = 1505	No of eyes n = 2978
Known (nonglaucomatous)	6	6
Expected (coecocentral)	18	23
Unknown and unexpected	38	46
False positives	14	16
Total	76	91

From our point of view the actual yield of the field screening was 46 *unknown and unexpected defects* – 18 glaucomatous and 28 "others" – described in Table II. They were all reproduced and also verified by Goldmann perimetry if not by ophthalmoscopy. Their extension was in general adequately mapped out by the automatic field chart.

Table II
Unknown and unexpected visual field defects

	No of cases	No of eyes
A Glaucomatous		
1 Arcuate	6	7
2 Arcuate and circumscribed	1	2
3 Circumscribed	8	9
Total	15	18
B Others		
1 Of unknown etiology*	2	2
2 Circumscribed paracentral – corresponding with various small but obvious fundus lesions	18	21
3 Homonymous paracentral	2	4
4 Bitemporal	1	1 *
Total	23	28

* One defect had disappeared at follow up – the other turned out to be part of a homonymous one

* Automatic perimetry was not attempted in the left eye in which the visual acuity was 2/60

The 18 glaucomatous defects were found in eyes with pathological [illegible] the disc All patients with glaucomas are of course followed up and a [illegible] account of the characteristics associated with glaucomatous field defects [illegible] present material is planned

The 28 others were caused by a wide variety of disorders [illegible] occlusions of small retinal veins (5 cases) and scars in retina and choroid [illegible] were most frequent Apart from the glaucomas only two cases with [illegible] defects (one chiasma compression and one retinal detachment) needed [illegible] care

The field screening was considered to be *false positive* in 14 cases (16 eyes [illegible] subjects could not be prevented from overfixating One deaf person [illegible] excellent lip reader and therefore by mistake left without adequate instruction [illegible] the dark room In six cases extraocular causes such as ptosis, blephar[illegible] smudged glasses and matted (super)cilia partly blocking the sight of [illegible] overlooked and therefore not eliminated Repeated telephone calls disturbed [illegible] individual and another suffered from migraine To sum up false positive [illegible] usually be given an adequate – though trivial – explanation Only one [illegible] had to be left unexplained and not verified by kinetic perimetry

The *blind spot* was missed" in 12% of all reliable tests This rate was [illegible] the fixation was good (i e the check light seen ≤2 times) and still less than [illegible] when the fixation check light was seen three to five times

The results in geographical subdivisions of the material were alike and the achievements reported at the beginning of the survey were well maintained [illegible] improved – at the end The rates of unreliable tests false abnormal tests and missed blind spots in repeated tests were similar to those in initial tests Unreliable tests were twice as common in men as in women The frequencies of missed blind [illegible] and false abnormal tests increased in the older men and decreased in the [illegible] women All types of true defects were more prevalent in the older half of the population

Discussion

When first recognizable glaucomatous field defects are small circumscribed [illegible] scotomas which may appear anywhere in the central field (Aulhorn & Harms 1967) Any attempt to detect progressive defects smaller than the blind spot [illegible] obviously be unprofitable since perimetry cannot be performed [illegible] perimeter treats the blind spot area in exactly the same way as the rest of the paracentral field The fact that the probability of detecting the blind [illegible] automatic perimetry is as high as about 0 9 should therefore mean that the [illegible]

amply fulfils any reasonable demands on *sensitivity*. Surely the detection of 46 unknown and unexpected defects does not contradict this conclusion.

The rate of *verified defects not warranting medical care* (2%) was greater than might be desired but of course a natural and inevitable consequence of the high sensitivity.

Our main concern was however *specificity* since a high rate of false positives might easily become a major obstacle. We were therefore much relieved to find that after retesting very few unexplained false positives remained.

We conclude that the procedure of automatic perimetry in the form applied is quick, sensitive, specific and dependable.

Acknowledgment

This work was supported by the Swedish Medical Research Council (proj. No. B79-04X-5909-09A) which is gratefully acknowledged.

References

Aulhorn E. & Harms H. (1967) Early visual field defects in glaucoma. In: *Glaucoma Symposium Tutzing Castle 1966*, pp. 151–186. Basel, New York: Karger.

Bengtsson B. & Krakau C. E. T. (1979) *Automatic perimetry in secondary prevention of glaucoma. A preliminary report.* (Nara Symposium 1978). In: glaucoma update. Springer Verlag, Berlin.

Heijl A. (1976) Automatic perimetry in glaucoma visual field screening. A clinical study. *Albrecht v. Graefes Arch. klin. exp. Ophthal.* 200, 21–37.

Heijl A. & Krakau C. E. T. (1975a) An automatic static perimeter, design and pilot study. *Acta ophthal. (Kbh.)* 53, 293–310.

Heijl A. & Krakau C. E. T. (1975b) An automatic perimeter for glaucoma visual field screening and control. Construction and clinical cases. *Albrecht v. Graefes Arch. klin. exp. Ophthal.* 197, 13–23.

Krakau C. E. T. (1978) Aspects on the design of an automatic perimeter. *Acta ophthal. (Kbh.)* 56, 389–405.

Authors' addresses:

Bo Bengtsson, med. lic., Dalby Community Care Research Centre,
Skolgatan 1, 240 10 Dalby, Sweden.

C. E. T. Krakau, professor, M.D., Department of Experimental Ophthalmology,
University Eye Clinic, 221 85 Lund, Sweden.

JUDICIA DE NOVIS LIBRIS

J. Bories The Diagnostic Limitations of Computerized Axial Tomography [illegible] Berlin Heidelberg New York 1978 175 figures [illegible] tables [illegible] pages Price [illegible] US dollars 27.–

The excellent method of computed tomography (CT scan) has completely [illegible] of modern neuroradiology. Accordingly the first wave of clinical [illegible] enthusiastic.

Of course there must be limitations to CT and it is nice to learn that CT [illegible] established that it is also open to criticism.

This is reflected by the present congress report (Europ. Soc. Neuroradiology [illegible] September 1976) in which the contributions focus not only on the established [illegible] but also on the diagnostic limitations of the method. A sound approach indeed [illegible] better differential diagnosis by CT – and leaving credit to the *combined* use of [illegible] methods (CT *and* other) with the most suitable combination to be chosen by the [illegible] according to the demands of the single case).

The 24 reports are grouped into five sections. The diagnostic limitations of CT in 1) cerebral tumours 2) diseases of the orbit and of the skull base and the face [illegible] infarts, oedema and subdural haematomas. Section 4) deals with CT and [illegible] radiological techniques and 5) with accuracy and further prospects of CT.

In part 2) the article on orbital problems (by Moseley et al. including also the [illegible] is of special interest to ophthalmologists.

Otherwise the book primarily addresses itself to neuroradiologists. It is [illegible] especially for those who have recently started working with CT but are not yet experienced with the possibilities of the technique.

[illegible]

K. Schuchardt and *N. Schwenzer* Fortschritte der Kiefer- und Gesichts-Chirurgie [illegible] buch Band XXIV. Plastische Chirurgie im Mund-, Kiefer- und Gesichts[illegible] Georg Thieme Verlag Stuttgart 1979 1[illegible]0 pages 148 illustrations Price DM [illegible]

This yearbook contains a series of brief discussions of various aspects of [illegible] surgery. Most of the techniques presented would seem not to be relevant to an [illegible] surgeon and only one paper can be said to fall within the field of traditional [illegible] surgery. This paper describes correction for blepharochalasis.

Although therefore one has to say that this book is of only limited [illegible] ophthalmologist it could as well be represented as a brief well illustrated and [illegible] survey of what neighbouring surgical specialties can offer the multi-traumatized [illegible] formed patient. With this background it can be recommended to [illegible] ophthalmologists.

[illegible]

F a c u ∽ A de Ro ck Electrodiagnosis Toxic Agents and Vision 1 th I S C E V Symp ium Ghent 19/7 Junk The Hague Boston London 19/8 343 pages Price Dutch Guilders 110 –

his book consists of 54 papers delivered at the 15th symposium of the International Society r Clinical Electrophysiology of Vision which was devc ted to electrophysiological findings nd diagnosis in connexion with toxic or other damaging effects on the retina the pigment pithelium the visual pathways and the visual cortex

Among the substances shov ing or tested for effects on ERC EOC or VEP componenets ere chloroquine thioridizine gentamicin diphenylhydantoine barbiturates urethane ak tane ethanol methanol zinc iron copper (metallosis bulbi) C ABA taurine ganglio- ides sodium iodate and neurotoxins Other damaging effects included photocoagulation rauma and ischaemic retinopathy

Toxicology of drugs and other substances is becoming increasingly important in clinical ractice as well as in research This book which gives a rather good picture of the field of lectroophthalmology may be warmly recommended to ophthalmc logists and ele trophysio ists interested in toxic effects on the visual system

S e F ik Nil on

d Junker Fri del Kontaktlinsen Praxis einer optimalen Anpassung Urban & Schwar zenberg Munchen Wien Baltimore 1978 140 pages 90 figures Price DM 54 –

early two per cent of the population in Germany wear contact lenses training in contact ens fitting is an educational routine in major eye departments This book addresses itself to he beginner who wants to learn practical fitting techniques Its synopsized st le does not ield much space for theoretical considerations but most of the text is de oted to practical neans Hard and soft lenses of classical or more recent composition (CAB sil one polycone are discussed as well as different designs of toric lenses A tabular summary of the commercially available lens types provided with the manufact r s fitting instructions and a list of the major brand contact lens care products with a des ription of their chemical const tution provide useful information

The author treats his subject in a reporting form which now and then lea es the reader withc ut a personal attitude based upon the author s practical experiences

H wever the book can be recommended either as an impetus to start or as a basic textbook for thc se ophthalmologists interested in the fine art of fitting contact lenses

I D ner

Klaus Heilmann Therapeutic Systems pattern pec fic drug delivery Concept and de el opment Georg Thieme Verlag Stuttgart 1978

The boc k deals with the principles of controlled or monitored drug del ery and the remarkable results Alza Corporation Palo Alto California, USA have achieved in this field

In the first part of the book, the author defines the biopharmaceutic principles forming the basis of the development of new therapeutic systems i e new ways of administrating drugs such as continuous drug release at a certain rate per time unit He discusses the limits of c nventional forms of drug administration and gives a short survey of possible membranes

and energy sources for new therapeutic systems. In the last part of the book [illegible] therapeutic systems now in use in medicine and as experimental [illegible]. It includes Therapeutic Systems for systemic use, Therapeutic Systems for local use [illegible] and osmotic minipump and a system with a so-called Closed [illegible] artificial beta cell).

For ophthalmologists the most interesting part is that describing the [illegible] therapeutic system. Here a broad and for the practising ophthalmologist [illegible] description is given beginning with the old Greeks. The book is a substantial [illegible] good introduction and understanding of pharmacokinetics and technology of [illegible]. This is of importance not only for all those using the Ocusert delivery system [illegible] medical practitioners. Knowledge and technology in these fields is increasing [illegible] one can expect to see a number of therapeutic systems developed and [illegible] medicine in the near future.

The book is easy to read with a number of good and instructive figures and [illegible] students and medical practitioners alike.

[illegible]

Augenärztliche Fortbildung. Jahreskurse für die praktische Augenheilkunde. Band 4, Teil 3. 1976. Herausgeber: Hanns Jürgen Merte. Urban & Schwarzenberg.

This book is the third in a series of four books designed for the postgraduate training of ophthalmologists.

Three different topics are treated in the book: traumatic retinal detachment [illegible] Gärtner), the neuro-opthalmology of eye muscle palsies, the differential diagnosis [illegible] (Alfred Huber) together with ophthalmoscopy (E. Kleberger).

The book is easily read and is well illustrated, but presents nothing new. The [illegible] without difficulty be found in the standard textbooks as Duke Elder or otherwise. The book is therefore hardly of interest to Scandinavian readers.

[illegible]

Glaucoma. Conceptions of a disease. Pathogenesis, Diagnosis, Therapy. Georg Thieme Verlag, Stuttgart. Distribution for USA by W. B. Sauders Company [illegible] Igaku Shoin Ltd., Tokyo. 434 pages, 32? figures, 93 tables, [illegible] colour pages. Price [illegible] 128,–.

As indicated by the title, the purpose of the book is to give a comprehensive survey of the glaucomas.

The book is divided into eight sections: 1) Glaucoma. Conceptions. 2) Functional [illegible] physiology and pathology. 3) Glaucoma damage. 4) Methods of examination. 5) [illegible] cology. 6) Management of the glaucomas. 7) Surgical techniques. [illegible]) The [illegible] Classification and synthesis.

Thirty-one acknowledged experts in the different fields of glaucoma from a total of [illegible] countries have succeeded in giving an inspiring description of the present day [illegible] of glaucoma. After a somewhat disappointing period with focus on the intraocular pressure, new advances are to be expected to be made in the two other areas of the [illegible] namely problems concerning damage to the optic nerve head and disturbances in the visual field.

For the reader who wishes to further supplement his knowledge of the glaucoma the book ends with 39 pages of references which satisfactorily cover the more recent publications up to the beginning of 1977

The book is of value to the ophthalmologist who wishes to have an up-to-date account concerning the problems of glaucoma, which continues to give rise to a most extensive stream of information.

Knud Nø skov

Berufsverband der Augenarzte Deutschlands Arbeitskreis *Kontaktlinsen.* Einfuhrungs und Fortbildungsvortrage der Arbeitskreistagungen, Wiesbaden 1966–1973 Edited by M Freigang 1976 (Nurnberg)

The book comprises a collection of reports of approximately 60 lectures given in Wiesbaden in the years between 1966 and 1973 The book contains many topics with the main stress laid on the technique of fitting contact lenses the method of using contact lenses in keratoconus astigmatism and aphakia as well as the fitting of contact lenses in children

The book also contains several sections on the physiological and pathological changes seen *with the use of contact lenses*

The book offers nothing new and can hardly be said to be of great interest It cannot replace the standard textbooks on contact lenses of which there is now a good selection

Y A Fahmy

E J Klein Die Schilddruse Diagnostik und Therapie ihrer Krankheiten nd ed Springer Verlag Berlin, Heidelberg New York. 1978 904 pages 61 ill Price DM 68 – or US *dollars 34 –*

The present edition is a completely revised up-to-date successor to the 10-year-old first edition with stress *on the medical endocrinological problems*

The diagnosis of endocrine ophthalmopathy can be a considerable problem for the ophthalmologist. *The most recent hormonal methods of examination are discussed It is* emphasised that a combination of several tests is necessary The metabolism can as is well known be normal or even reduced in endocrine exophthalmus T_3 will be relatively raised in relation to T_4 The radio-iodine uptake curve over the thyroid gland is raised Suppression with T_3 will be negative Thyrotropine releasing hormone will not increase the thyrotropine blood level

These tests and their limitations are discussed on the basis of about 4000 patients with all types of thyroid *disorders of these 1500 had an ophthalmopathy*

During treatment great importance is attached to preventing hypophyseal induced exophthalmus (antithyroid treatment combine with steroid and thyroid hormone) The difficulty in diagnosis and therapy is demonstrated

Positive autoimmune reaction L A T S D-thyroxine roentgen treatment of the apex of the orbit, etc are considered

The book is well written and well illustrated with photographs of patients and instructive diagrams It may serve *to further the co-operation* between the intern medical specialist and the ophthalmologist

M S Norn

Third International Conference on Myopia

The International Society for Myopia Research announces the Third International Conference on Myopia to be held in Copenhagen, Denmark, 24th to [illegible] th August [illegible]
Open to members of the Society and others actively involved in myopia research.
Official language: English
Abstracts should be submitted before March 1st, 1980
For further information and abstract sheets contact: Third International Conference on Myopia, University Eye Clinic, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen [illegible]
Persons interested in joining the Society should contact the secretary: Francis A. Young, Ph.D., International Society for Myopia Research, P.O. Box 2099 C.S., Pullman, Washington 99163, U.S.A.

Augenklinik der Medizinischen Hochschule Hannover (H[illegible] H. H[illegible]gger)

SLIDING OF EPITHELIUM IN EXPERIMENTAL CORNEAL WOUNDS

A scanning electron microscopic study

BY

HORST BREWITT

The morphological changes in the rabbit cornea due to a linear wound and a central keratectomy and its reepithelization were examined under the scanning electron microscope. After the rounding off and retraction of the edges of the wound during the first 6 h the stroma was infiltrated by leucocytes. First of all the superficial epithelial cells migrated over the edges of the wound to protect the exposed cleft in the tissue. After 15 h the basal epithelial cells sent pseudopodia like processes of varying lengths into the wound area. The migrating cells were mainly flat. During this phase the primary migration of the basal epithelial cells was followed by subsequent cell layers. In the migration three types of cells can be distinguished: 1. very flat cells with smooth edges without microprojections which adapt their shape to the clefts in the damaged stroma, 2. flat cells with considerably folded plasma membrane on the cell surface giving a coral like appearence, 3. cells with voluminous cytoplasmic processes. The rapidly sliding epithelium covered the defect with star shaped growths so that after only 48 h a nearly complete closure of the wound was achieved. After 96 h the deep wound was completely covered by epithelium. The epithelial cells did not yet possess their original surface structure in that they had demonstrably fewer microvilli and microplicae than normal. Only after five days at the earliest could the normal surface relief be seen. The surface cells were predominant in the first stage of the healing process and the basal cells only in the second stage.

Key words: cornea – corneal epithelium – epithelial sliding – wound healing – scanning electron microscopy – rabbit.

The healing of a corneal wound is accomplished through two processes. Firstly through a sliding movement and then by a multiplication of epithelial cells. The independent amoebic movement of the epithelial cells was first described by Peters

Received May 2 1979

(1885) This was subsequently confirmed by the classic investigations of [illegible] wald & Buschke (1944) Buschke (1949) Heydenreich (1958) and [illegible] (1960) Experimental wound healing as described by several authors [illegible] involves extensive wounds and various complicating factors rather than the detailed movement of the epithelium

The exact morphological presentation of the possible cell movements has [illegible] now been limited by the resolution of the light microscope The scanning electron microscope makes it possible to examine the cell surface at higher magnification Pfister (1975) has for the first time used this technique to examine the healing [illegible] purely epithelial wounds

The purpose of our study was to demonstrate in detail the behaviour of [illegible] epithelial cells and their morphological changes during the repair phase of [illegible] healing of deep corneal wounds

Materials and Methods

Thirty-one fully grown rabbits with healthy eyes were used. The animals were anaesthetised with an intramuscular injection of Ketamin (Ketanest® [illegible] 25 mg/kg body weight) and Xylazin (Rompun® Bayer 0 2 mg kg body weight

Group A (10 rabbits) Using a scalpel with the aid of a surgical microscope a [illegible] but non penetrating cut of approximately 5 mm length and 0 4 mm depth was made The corneas were then examined after 0 1 3 6 and 15 h

Group B (21 rabbits) With the aid of a surgical microscope a central lesion of [illegible] depth was made using a 2 5 mm trephine The epithelium and [illegible] the circular lesion were removed with a hockey knife The corneas were examined after 0 6 15 24 48 96 and 120 h The animals were killed with an [illegible] monary injection of 20% N (2 (m Methoxyphenyl)-2 aethyl-butyl)-(1) [illegible] butyramid + 5% 4 4 Methylen bis (cyclohexyl trimethyl ammonium [illegible] 4 Butylaminobenzol 2-dimethylamino-aethanolhydrochlorid (T 61® [illegible] ml) and the corneas were immediately irrigated for 5 min with 4% [illegible] in 1/15 M Sørensen s phosphate buffer (pH 7 2) before the corneas were [illegible] using a 9 mm trephine They were then treated according to Pfister ([illegible] washing with 20% acetylcystein (Mucolyticum Lappe® *Lappe*) for 10 [illegible] again for 10 min then washed in 20% acetylcystein and finally fixed for [illegible] h After washing the preparation in Sørensen s phosphate buffer for 1 h they were further fixed in osmium tetroxide in phosphate buffer and dehydrated in [illegible] ing conentrations of acetone The dehydration of all preparations was [illegible] using critical point drying All preparations were coated with a 20 nm [illegible] examined in a Stereoscan 600 (Cambridge)

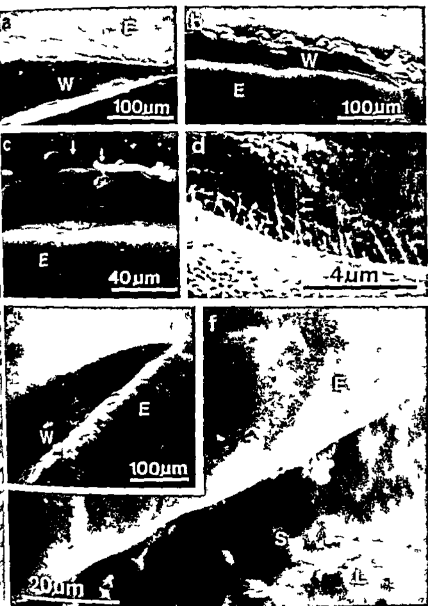


Fig 1

a) Zero-time after linear wounding The wound opening (W) is wide and the wound edges are retracted. Epithelium (E) (240×)
b) 1 h after linear wounding Detached epithelial cells in the wide wound (W) Epithelium (E) (240×)
c) 1 h after linear wounding Detached epithelial cells (arrows) the wound edge is covered with sliding epithelium (E) (480×)
d) 1 h after linear wounding Filopodia (arrows) extend out toward the wound (7250×)
e) 3 h after linear wounding The wound opening (W) is wider and the edges are covered with epithelium (E) (240×)
f) 3 h after linear wounding Sliding epithelium (E) has covered the wound edge Leukocytes (L) invade into the stroma (S) (1450×)

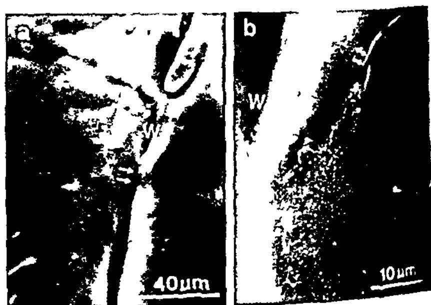
Results

Group A

Zero time after corneal wounding (4 eyes) There is a clear retraction of the wound edges and the whole wound gapes open (Fig 1a) At about the same time the epithelial cells round off and the stroma fibers appear to shrink correspondingly

1 h after corneal wounding (4 eyes) At the edge of the wound are detached cells which were damaged during the wounding and are now shed (Fig 1b) Superficial cells slide under the damaged cells over the rounded wound edge (Fig 1c) These cells possess densely accumulated microvilli and microplicae and extend cell processes into the wound area (Fig 1d)

3 h after corneal wounding (4 eyes) As before a gaping wound can be seen The superficial epithelial cells are however already spread over a large part of the stroma (Fig 1e) They display a regular complement of microvilli and on their surfaces For the first time leucocytes are now found in the wound (Fig 1f)



Fig

6 h after linear wounding

a) In some areas the wound edges have already adhered (arrows) ... (180×)

b) The wound edge (W) is covered with sliding epithelium the epithelial ... interdigitation (arrows) (1 800×)

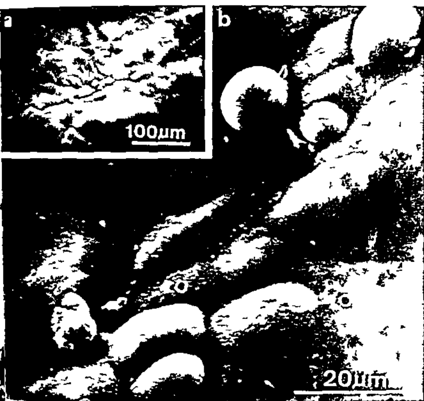


Fig 3

15 h after linear wounding

On nal wound (arrows) is filled with epithelial cells (145 ×)

Epithelial cells in the original wound area are interdigitated with each other and appear ressed and rounded off (1450 ×)

h *after corneal wounding (4 eyes)* The wound slit is narrower In isolated areas the und edges have already adhered (Fig 2a) The superficial cells are also found p within the wound cleft. Outside the actual area of the wound many dark thelial cells are apparent some of which have no regular microstructure These s interact loosely with each other (Fig 2b)

5 h *after corneal wounding (4 eyes)* The wound slit has closed and can only be ecognised by the epithelial structure (Fig 3a) The epithelial cells in this area ppear compressed rounded off and partially overlap one another (Fig 3b) They lso have a dense microstructure on their surface This indidcates an excessive pithelisation In other regions in the previously damaged area very narrow p helial cells are seen whose elongated axis lie parallel to the wound slit.

Group B

Zero time after deep corneal lesion (6 eyes) The whole wound edge appears ... (Fig 4a) Detached epithelial cells lie at the wound edge while the ... lamellae and open tissue lesion remain exposed (Fig 4b)

6 h after deep corneal lesion (6 eyes) In the bed of wound large numbers of ... are found (Fig 4c) The original vertical edge of the wound is ... (Fig 4d) The superficial layer of epithelial cells which can be traced ... undamaged epithelium into the bed of the wound has slid over the ... edge

15 h after deep corneal lesion (6 eyes) Striking in this phase are the ... club like cell processes which extend themselves from the superficial cells ... actual migration front out over the stroma (Fig 5a) They are ... and 5–8 µm wide These processes possess numerous short ... For the first time the basal cells can also be seen These spread up from ... rounded and superficial cell covered wound edge

24 h after deep corneal lesion (6 eyes) About one half of the original bed of the wound is epithelised Different forms of migrating epithelial cells are now found (Fig ...) Sometimes these cells possess smooth cell edges which nestle to the corneal ... In other cases the cell walls of the migrating cells show a coral like appearance ... impression originates in this case from the fact the cell projections ... perpendicularly to the area of the wound

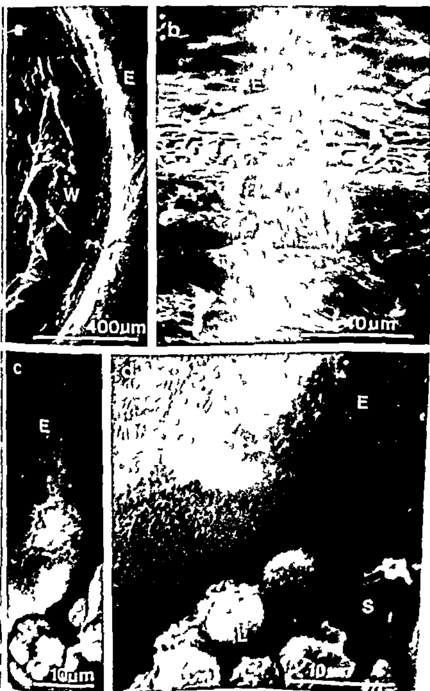
48 h after deep corneal lesion (6 eyes) A nearly complete epithelisation ... to a small central region of approximately 250 µm diameter (Fig 7a) The ... wound edge is still clearly visible Leucocytes are no longer seen in the ... region The epithelial projections which have slid from the ... wound edge to the centre interdigitate with one another (Fig ...) ... migration takes place from all sides an impression of excessive epithelial ... sometimes given Cells project out over the surface (Fig 7a)

Fig 4

a) Zero time after deep corneal lesion Retracte... (... of stroma corneae (72 ×)

b) Zero-time after deep corneal lesion Detac... (72 ×)

c and d) 6 h after deep corneal lesion The ... slid over the wound edge Leucocytes (L) ... Fig 4d 2000 ×)


a
E
W
400μm
b
E
10μm
c
E
10μm
d
E
S
L
10μm

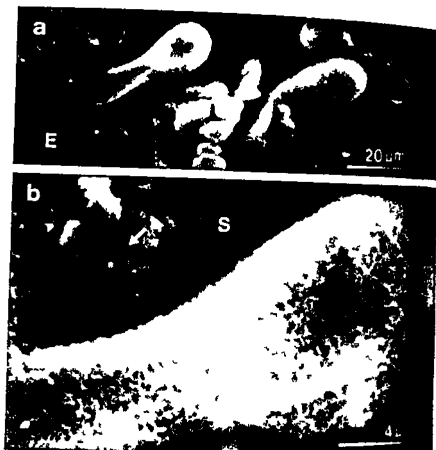


Fig. 5

1 5 h after deep corneal lesion

a) Voluminous cell processes extend from superficial epithelial cells (E) over the corneae (S). Leucocytes (L) (1450×)

b) Cell process with short microplicae basal epithelial cell (arrow). Stroma (7250×)

Fig. 6

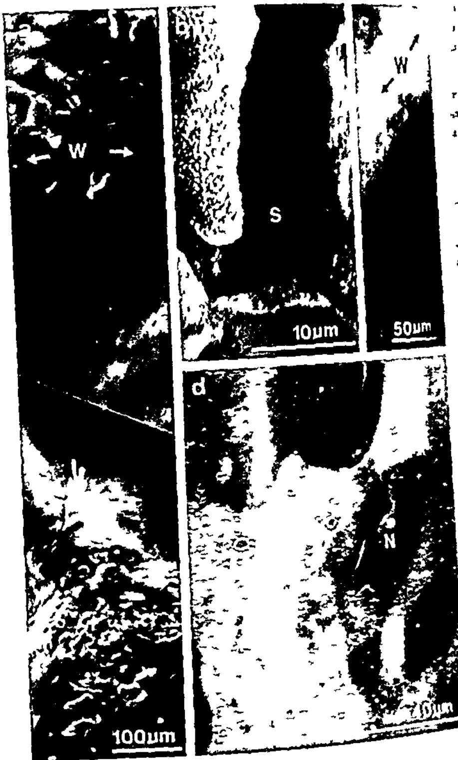
24 h after deep corneal lesion

a) Sliding epithelium (E) with different forms of migrating cells. Stroma (2000×)

b) Flat cell with a microvillous free zone (1) and cell with a ... ral like appearance

c) Ruffles of cell margin sometimes perpendicular to the cell surface (arrow)


a
b
c
S
S
S
S
E
10µm
4µm
4µm


W
b
S
10µm
c
W
50µm
d
N
10µm
100µm

96 h after deep corneal lesion (6 eyes) The original region of the wound of 2 5 mm diameter is completely covered with a layer of epithelium (Fig 7c) The epithelial cells of this region appear largely dark and the contours of the cell nuclei can be seen (Fig 7d) In some places disruption of the cell association occurs

120 h after deep corneal lesion (6 eyes) The original lesion is still recognisable as a depression The surface relief of the epithelial cells has reached an almost normal condition

Discussion

The sliding of the epithelial cells is a common feature seen in wound healing of the cornea. This investigation has been able to demonstrate morphological detail relating to the sliding cells Initially the whole wound edge retracts slightly so that the superficial cells of the corneal epithelium can migrate over the whole wound e within the first 6 h after wounding When the stroma has also been damaged the cells must slide not only over the open cut in the epithelium but also over the left in the stroma The closure of the wound edge perpendicular to the surface is ieved through additional enlargement of the surface of these cells which under normal conditions are very flat This sealing of the wound periphery protects the subsequently regenerating basal cells from noxious substances The cells appear to draw their potential for expanding their surface from the otherwise densely accumulated microprocesses since during the migratory phase these cell processes are sparser in the covering of large wound areas than in that of a narrow cut After approximately 15 h a transition phase which is conspicuous in extended wounds is reached In some areas the movement is still led by superficial cells while in other regions of the wound edge basal cells are already moving out from under the superficial cells

After 24 h the epithelisation of the heavily damaged stroma is finally taken over by the basal cells which are then followed by further cell layers The sliding cells adapt their shape to that of the tissue cavities Because of the damage to the basement membrane the adhesion to the epithelium is poor and only after 96 h can

---

Fig 7

a) 48 h after deep corneal lesion The area of the original defect is nearly complete epithelised up to a small residual defect The original wound edge (W) can be noted (145×)

b) 48 h after deep corneal lesion Central area of the residual defect Epithelial cells stroma corneae (S) (2900×)

c) 96 h after deep corneal lesion The original wound is completely covered with epithelium original wound edge (W) (1455)

d) 96 h after deep corneal lesion The surface cells are predominantly large and dark with visible nuclei (N) (495×)

the *epithelium* close a lesion of 2 5 mm diameter *The* superficial [illegible] epithelial cells is very different from its normal state emphasizing [illegible] epithelised *wounds* are very vulnerable The intercellular connections [illegible] the microprocesses of the plasma membrane are only poorly defined The irregularities of the whole epithelium cause the precorneal film to [illegible] and the appearance of dehydration in *some* areas is possible *The* very [illegible] with visible nuclei are dehydrated *cells* Disruption of the epithelium [illegible] any time up to this point The surface *relief* of *the* sliding epithelial [illegible] approached that seen under *normal conditions* only after 120 h. The [illegible] epithelium can now perform its function of sealing the tissue The [illegible] nishment of the substances lost from the *stroma* corneae can then proceed [illegible] this protective epithelial *cover In* the first phase of wound healing [illegible] multilayered epithelium behaves in principle as the single layered [illegible] (Schierholter & Honegger 1975 Nelson & Revel 1975 Hirsch et al [illegible] Horn et al 1977) The *sliding* movement to cover the tissue defect appears [illegible] unique nature of the corneal epithelial and endothelial cells.

In this study (as in the studies using the transmission electron [illegible] (Kuwabara *et al* 1976)) *the* first cell movements were seen after 1 h. What [illegible] induces *the epithelial cells* to slide in the described fashion is as yet not clear The question why *sliding* cells possess at one time smooth or wave-like cell [illegible] another *time send out* coral like or pseudopodia like processes cannot be [illegible] with the help of the scanning electron microscope Microfilaments as well as intracellular pressure are reported to play a role in the sliding movement ([illegible] et al 1973 Dispasquale 1975 Wohlman & Allen 1968 Taylor et al 19[illegible] Anderson 1977 *Gipson* 1977) Intracellular glycogen provides the [illegible] energy *for the* cell movement (Henninghausen et al 1972 Thoft & Friend [illegible])

In purly epithelial wounds the stability of the association between the epithelial cells is regained after a week at the latest (Pfister 1975 Haik & Zimm 1977 [illegible] 1976) If however the basement membrane is also damaged the adhesion between the epithelial layer and the stroma corneae is achieved later even though the regeneration of the basement membrane probably begins shortly after the [illegible] tion of *the* epithelial cells starts The first evidence of regenerated basement membrane using the transmission electron microscope was found between [illegible] 19 days (Khodadoust et al 1968 Blumcke et al 1969 Pulhorn & Thiel [illegible] Kenyon et al 1977) The results using the scanning electron microscope show clearly the loose connection between the sliding epithelial cells and the [illegible] underline the importance of the basement membrane

In summary the main role of the epithelium in reparative processes of the [illegible] is the restoration of the integrity of the protective epithelial cover This is [illegible]

most rapidly by sliding which enables the superficial cells with appropriate organisation to protect the cells of the stratum germinativum from further damage The basal cells can then take over the control of the repair of the damage

Acknowledgment

This investigation was realized in cooperation with the Institut fur Elektronenmikroskopie der Medizinischen Hochschule Hannover The author is greatly indebted to Prof Dr E Reale for advice

References

Blumcke S Rode J & Niedorf H R. (1969) Formation of the basement membrane during regeneration of the corneal epithelium *Z Zellforsch 93* 89–92

Buschke W (1949) Morphologic changes in cells of corneal epithelium in wound healing *Arch Ophthal (Chicago) 41* 306–316

Dipasquale A (1975) Locomotion of the epithelial cells *Exp Cell Res 95* 425–439

Friedenwald J S & Buschke W (1944) The influence of some experimental variables on the epithelial movement in the healing of corneal wounds *J cell comp Physiol 23* 95–107

Gipson I K (1977) Cytoplasmic filaments Their role in motility and cell shape *Invest Ophthal 16* 1081–1084

Gipson I K & Anderson R. A. (1977) Actin filaments in normal and migrating corneal epithelial cells *Invest Ophthal 16* 161–166

Haik B G & Zimny M L (1977) Scanning electron microscopy of corneal wound healing in the rabbit *Invest Ophthal 16* 787–796

Hanna C & O Brien J E. (1960) Cell production and migration in the epithelial layer of the cornea *Arch Ophthal (Chicago) 64* 536–539

Hennighausen U Schmidt Martens F W & Reim M (19 9) Metabolitspiegel und Enzymaktivitaten des energieliefernden Stoffwechsels im regenerierenden Corneaepithel *Ber Dtsch Ophthal Ges 71* 95–99

Heydenreich A (1958) Die Hornhautregeneration In Sammlung zwangloser Abhandlungen aus dem Gebiete der Augenheilkunde Heft 15 VEB Carl Marhold Verlag Halle

Hirsch M Faure J P Marquet O & Payrau P (1975) Regeneration de l endothelium corneen chez le lapin Etude microscopique et relation avec l epaisseur de la cornee *Arch Ophtal (Paris) 35* 269–278

Kenyon K R Fogle J A Stone D L & Stark W J (1977) Regeneration of corneal epithelial basement membrane following thermal cauterization *Invest Ophthal 16* 292–301

Khodadoust A A Silverstein A M Kenyon K R & Dowling J E (1968) Adhesion of regenerating corneal epithelium *Amer J Ophthal 65* 339–349

Kuwabara T Perkins D G & Cogan D G (1976) Sliding of the epithelium in experimental corneal wounds *Invest Ophthal 15* 4–14

Lemp M A (1976) Cornea and Sclera *Arch Ophthal (Chicago) 94* 473–490

Nelson G A & Revel J P (1975) Scanning Electron Microscopic Study of Cell Movements in the Corneal Endothelium of the Avian Embryo *Dev Biol 42* 315–333

Peters (1885) Die Regeneration des Epithels der Cornea. Inaug Dissert. ... (1910) Experimentelle Untersuchungen uber die Regeneration ... unter normalen Verhaltnissen und unter therapeutischen Maßnahmen ... Arch Ophthal 75 350–400
Pfister R R (1973) The normal surface of corneal epithelium ... microscopic study Invest Ophthal 12 654–668
Pfister R R (1975) The healing of corneal epithelial abrasions in the rabbit ... electron microscopic study Invest. Ophthal. 14 648–661
Pulhorn G & Thiel H J (1974) Ultrastrukturelle Untersuchungen zur ... Basalmembran des Hornhautepithels. Albrecht v Graefes Arch. klin exp Ophthal ...
Schierholter R & Honegger H (1975) Die Morphologie des Hornhaut... normalen Bedingungen und in der Regeneration nach mechanischer ... Advances in Ophthalmology Vol 31 Roper Hall M J Sautter H and Streiff E B eds ... 34–99 Karger Basel
Taylor D L Condeelis J S Moore P L & Allen R D (1973) The contractile ... ameboid movement I The chemical control of motility in isolated cytoplasm J Cell ... 59 378–394
Taylor D L Rhodes J A & Hammond S A (1976) The contractile basis of ... movement II Structure and contractility of motile extracts and plasmalemma ... ghosts J Cell Biol 70 123–143
Thoft R A & Friend J (1977) Biochemical transformation of regenerating ... epithelium Invest Ophthal 16 14–20
Van Horn D L Sendele D D Seideman S & Buco P J (1977) Regenerative ... corneal endothelium in rabbit and cat Invest Ophthal. 16 597–613
Wohlman A & Allen R D (1968) Structural organization associated with ... extension and contraction during cell locomotion in Difflugia J Cell Sci 3 105 ...

Author's address

Priv Doz Dr med Horst Brewitt Augenklinik der Medizinischen Hochschule Hannover Karl Wiechert Allee 9 D 3000 Hannover 61 Federal Republic of Germany

Prause J U Sjostrom H Noren O & Josefsson L. (1978) A micromodification of the electroimmunoassay *Ann. Biochem 85* 564–571
Schirmer O (1903) Studium zur Physiologie und Pathologie der Tranenabsonderung und Tranenabfuhr *Arch. Ophthal (Chicago) 56* 197–991

Author s address
J U Prause M D Eye Pathology Institute
Frederik V s Vej 11 5 DK 2100 Copenhagen Ø Denmark

Department of Ophthalmology (Head: Salme Vannas), University of Helsinki, Helsinki, Finland.

HISTOCOMPATIBILITY AND CORNEAL GRAFT ENDOTHELIUM

BY

PEKKA RUUSUVAARA

Endothelia of 102 clear human corneal grafts in 95 patients were photographed *in vivo* with a specular microscope. The relation of the endothelial cell density to the HLA and ABO compatibility of the donor recipient pairs were studied. In addition, the effects of vascularization and reversible rejection on the endothelial cell density were analysed.

A negative correlation was found between the HLA incompatibility and the endothelial cell density of the graft. The group with 0–1 (2) mismatches had the highest endothelial cell density (1356 ± 510 cells/mm²). This result was better than in the group with 2 (3) mismatches, but the cell difference was not statistically significant. A statistically significant difference in average endothelial cell densities was found between the groups with 0–1 (2) mismatches and with 3–4 mismatches, the latter having 880 ± 475 cells/mm² ($P < 0.01$). There was also a statistically significant difference in average endothelial cell densities between the group with 0–1 mismatch and the untyped group ($P < 0.01$). The difference in cell densities between the group with 2 (3) mismatches and the more incompatible groups together (3–4 mismatches and the untyped group) was statistically almost significant ($P < 0.05$).

The ABO-compatible pairs had 18% more endothelial cells than the ABO-incompatible pairs. The difference was not statistically significant, but when the ABO-compatible pairs were compared with all the others (incompatible and untyped), the difference in mean cell density between the two groups was statistically almost significant ($P < 0.05$).

Comparison of the effect of tissue incompatibility on graft endothelial cell density separately in the patients with vascularized and non-vascularized

Received March 2[illegible]

corneas showed that in both categories the endothelial cell density was highest in the group with 0 – 1 (2) mismatches

In 8 patients with a reversible rejection episode the average cell density was °0% less than in those with an uneventful postoperative period The importance of rejection on the endothelium in some special cases is discussed

Key words cornea – corneal graft endothelium – graft rejection – histocompatibility – corneal vascularization – specular microscopy

In efforts to obtain a clear and functioning corneal graft even in difficult vascularized cases ophthalmic surgeons have chosen the most viable material available from young donors The immunological causes of graft failure in non vascularized cases have been neglected Most authors believe that the immunological privilege of the cornea makes matching of donor and recipient tissues of reduced value in corneal grafting (Allansmith et al 1974) On the other hand there is some evidence that in the human cornea the immunological privilege is relative rather than absolute (Jones 1973 Polack 1973 Batchelor 1976 Vannas et al 1978)

Reports of the influence of ABO incompatibility on the results of corneal transplantation are inconclusive But among the ABO-compatible transplantations clear grafts were significantly more frequent among the more HLA-compatible than among the less HLA-compatible grafts (Ehlers & Kissmeyer Nielsen 1973)

This series was not large and the number of good matches (i e 3–4 shared HLA antigens) was very small Gibbs et al (1973) observed indications that in grafts transplanted to vascularized corneas greater HLA incompatibility was associated with a higher risk of rejection

In an earlier paper (Vannas et al 1976) we studied the importance of histocompatibility in corneal grafting using HLA typed donor recipient pairs We showed that in grafts with compatible donor material (0–1 mismatch) rejection occurs only exceptionally as compared with incompatible material We have therefore continued to select tissue typed cryopreserved corneas for tranplantations The purpose of HLA matching is to minimize graft incompatibility *Donor antigens that* are shared by recipient will obviously not harm the graft but antigens possessed only by the donor may possibly lead to rejection

The essential point for the survival of the graft endothelium may possibly be its HLA and ABO compatibility Destruction of the endothelium is the first of a series of clinical changes in the graft that culminate in its opafication

Earlier studies with the specular microscope have not shown any significant difference in *endothelial cell density between non rejected* and rejected but still partially clear corneal grafts (Bourne & Kaufman 1976b Sato 1978)

In this study specular photography has been used for the first time to measure

whether there is a correlation between the endothelial cell density of the cornea graft and the degree of histocompatibility between donor and recipient. In addition the effects of corneal vascularization and reversible graft rejection on endothelial cell density have been analysed.

Patients and Methods

Patients. The series presented comprised 102 penetrating keratoplasties in ... patients. Of these graftings 7 were bilateral and 5 were regraftings. The follow-up study was made in 1976–78. The follow-up periods varied from 1 month to ... years, averaging 3 years. The patients ranged in age from 16 to 77 years and the donors from 8 to 69 years. Among the patients the ratio of men to women was ... 40.

Operative method. The operations were performed mostly by the same two surgeons (SV and KK). The graft sizes varied from 7 to 8 mm. As suture material ... nylon monofilament was used for continuous or single sutures. In the earlier cases 9-0 virgin silk sutures were also used.

Postoperatively as prophylaxis all patients received corticosteroids ... in small doses perorally. When the cornea was heavily vascularized or signs of rejection could be seen the patient was also treated with immunosuppressive ... (Imurel[R])

HLA typing and ABO blood groups. HLA antigens were determined from peripheral blood lymphocytes of the recipient and of those donors who had been tied ... alive. Usually lymph nodes taken at autopsy provided viable lymphocytes for HLA typing of necro-donors. Typing was done routinely by the microlymphocytotoxicity technique (Amos et al. 1969), each HLA specificity being represented by 2– ... parallel antisera of local and/or international origin. Corneal donor-recipient pairs designated by 0–1 (2) or 2 (3) mismatches means that there may have been 2 or ... mismatches owing to the possibility of one undetected antigen. When only 3 HLA antigens are found the probable reason is homozygosity.

According to the degree of histocompatibility, i.e. the number of mismatches between donor and recipient, the series was divided into four groups. The first group contained 44 grafts with 0–1 (2) HLA mismatches, the second group ... grafts with 2 (3) mismatches and the third group 11 transplants with ... mismatches. The last group comprised 25 untyped donor eyes.

The ABO blood group was determined in 80 donors of these ... we...

Table I

Endothelial cell densities ± SD in transplants of different diagnostic groups at follow up

Diagnosis of patients	Number of cases/ vascularized corneas	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (year)	Endothelial cell density (cells/mm²)
eratoconus	60/ 3	(55) 48 5	33 5	1080 ± 4 8
ther dystrophies	8/ 3	53 0	5 6	994 ± 393
erpes	7/ 6	45 9	37 1	1179 ± 198
uberculosis or hlyctenulosa	3/ 1	46 0	66 7	1133 ± 496
philis or arenchymatosa	9/ 7	(8) 47 4	4 8	13 8 ± 535
ther inflammations	11/10	0 0	8 1	1554 ± 590
orrosion trauma r unknown	4/ 4	58 3	46 3	1344 ± 600

ompatible and 11 incompatible with the recipient and 22 donors were untyped The series of graftings was divided into three groups according to ABO compat bility

Donor material The donor series has been analysed in an earlier paper (Ruusuvaara 1979) according to corneal preservation donor age cadaver time and postope rative period Three methods of preservation has been used to store the donor corneas Altogether 41 corneas were used fresh or after moist chamber storage A total of 42 corneas had been cryopreserved (Capella et al 1965 1972) and 19 had been used after intermediate term preservation in MK medium The proportion of MK medium stored corneas in each compatibility group was about 20% The observation periods were about 5 years for the 3–4 mismatches and untyped pairs and rather less than 2 years for the more compatible pairs

Endothelial cell counting The specular microscope (Seyber Inc) was used to photo graph the central corneal graft endothelium (Laing et al (1975) Bourne & Kaufman (1976a)) The magnification of the negative was × 100 and the final magnification of the positive × 500 The endothelial cell population was counted from an area corresponding to 0 01 mm² on the endothelium In most cases counts were made from three to five different micrographs and the mean was taken as the result

Diagnosis and vascularization of the recipient cornea Table I shows the numbers of recipients in the different diagnostic groups and the proportions with vascularized corneas The largest diagnostic group (N = 60) consisted of patients with keratoconus only three corneas in this group were vascularized The other dystrophies comprised Salzmann s Groenouw s and Fuchs dystrophies of these eight corneas three were vascularized So these two groups consisted mostly of avascular recipient corneas The other patients had postinflammatory manifestations In nearly all the cases of postinflammatory changes and trauma the recipient cornea were vascularized

In most of the recipients the limbus had been investigated by fluorescein angiography before the operation Corneas were classed as vascularized if ghost vessels or one or more larger vessels could be seen According to this division 34 recipient corneas were vascularized and 68 non vascularized (Table I)

Rejection Eight patients had had a reversible rejection episode before photographs but their corneas were clear enough for specular micrography Thus it was possible to study the effect of the rejection episode on the endothelial cell density of the graft

Statistical analyses were made by Student s *t* test to ascertain the effects of ... factors on the parameters studied

Results

Recipient diagnosis and graft endothelial cell density

The average endothelial cell density of the 102 transplants was 1169 ± 418 cells/mm² with a mean donor age of 49 1 years

The recipients with keratoconus had a mean endothelial cell density of 1060 ± 458 cells/mm² in the graft The mean endothelial cell densities for recipients with postinflammatory corneal diseases are to be seen in Table I

Effect of HLA incompatibility on graft endothelial cell density (Table II)

Analyses of the effect of tissue incompatibility on the graft endothelial cell density indicated that the differnece in mean endothelial cell density between the 0–1 (?) and 2 (3) mismatch groups was more than 200 cells/mm² but statistically the difference is not significant Statistical significance in average endothelial cell densities was found between the groups with 0–1 (2) mismatches and with 3–4 mismatches (N = 11) the latter having 880 ± 475 cells/mm² ($P < 0\ 01$) There was also a statistically significant difference in average endothelial cell densities between

Table II

Effect of HLA incompatibility on the endothelial cell density of the transplants

HLA incompatibility (mismatches)	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cell mm)
0–1 (9)	44	(42) 48 3	4[illegible] 1	13[illegible] ± 510
2 (3)	22	(21) 50 8	40	1146 ± 413
3–4	11	(11) 47 5	38 3	850 ± 475
Untyped	25	(22) 49 7	40 [illegible]	989 ± 343
Total	102	(96) 49 1	40 8	1169 ± 478

Table IIa

Effect of HLA incompatibility on the endothelial cell density of transplant patients with keratoconus and with other diseases

HLA mismatches	Conus or other DG	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cell mm)
0–1 (09)	conus	24	(23) 48 8	34 3	1[illegible] ± [illegible]
	others	20	(19) 47 6	51 4	[illegible] ± 49
2 (3)	conus	14	(13) 52 3	31 [illegible]	1087 ± 4[illegible]
	others	8	(8) 48 3	5 8	1248 ± 290
3–4	conus	6	(6) 41 7	33 8	[illegible] ± [illegible]31
	others	5	(5) 54 6	43 6	1100 ± 621
Untyped	conus	16	(13) 47 2	33 6	1003 ± 381
	others	9	(9) 53 4	51 9	8[illegible]5 ± 283
Total	conus	60	(5[illegible]) 48 5	33 [illegible]	1080 ± 458
	others	42	(41) 49 9	51 4	129[illegible] ± 484

the group with 0–1 (2) mismatches and the untyped group ($N = 95$, $P < 0.01$) The difference in cell density between the group with 2 (3) mismatches and the more incompatible groups together (3–4 mismatches and the untyped group) ($N = 56$) is statistically almost significant ($P < 0.05$)

Table IIa shows that the importance of histocompatibility is almost as clear among the patients with keratoconus as among those with other diagnoses and with a higher proportion of vascularized corneas In both groups the same tendency was observed for endothelial cell density to decrease with increasing incompatibility

Effect of vascularization on graft endothelial cell density (Table III)

All corneas with blood vessels in the FAG of the cornea were regarded as vascularized The vascularized corneal bed group comprised 34 transplants with a mean endothelial cell density of 1297 ± 469 cells/mm² The non vascularized corneal group comprising chiefly corneal dystrophies with a total number of 68 transplants had a mean cell density of 1106 ± 474 cells/mm² The difference in cell density between the vascularized and non vascularized groups was statistically almost significant ($P < 0.05$)

The relative importance of HLA incompatibility and vascularization (Table IV)

The patients were divided first according to vascularization into those with non vascularized and vascularized corneas and then into subgroups according to tissue incompatibility In both vascularized and non vascularized groups the compatible grafts had clearly more endothelial cells than the incompatible or untyped ones Among the patients with vascularized corneas no difference was found

Table III
Endothelial cell density of transplants in vascularized and non vascularized recipient corneas

Recipient corneal bed	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells mm²)
Vascularized	34	(34) [illegible]	48 9	1297 ± 469
Vascularized	68	(69) 48 4	37 1	1106 ± 474
Total	102	(96) 4 0	40 8	1169 ± 4 8

Table IV

Effect of HLA incompatibility on the endothelial cell density of the transplants in patients with vascularized and non vascularized corneas

HLA incompatibility (mismatches)	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells mm²)
Vascularized				
0–1 (2)	16	(16) 45 9	48 6	1491 ± 591
2 (3)	7	(7) 51 4	56 1	1223 ± 293
3–4	4	(4) 51 8	56 5	1269 ± 570
Untyped	7	(7) 58 7	46 6	944 ± 178
Total	34	(34) 50 4	48 3	1297 ± 469
Non-vascularized				
0–1 (2)	28	(26) 49 7	38 3	12[illegible]9 ± 4[illegible]
2 (3)	15	(14) 50 4	33 1	1109 ± 463
3–4	7	(7) 45 1	39 3	658 ± 235
Untyped	18	(15) 45 5	37 7	100[illegible] ± 392
Total	68	(62) 48 4	37 1	110[illegible] ± 4[illegible]4

between the groups with 2 and with 3–4 mismatches However these groups comprised only 7 and 4 patients which is too small a number for statistical analysis In the non vascularized group the difference between the groups with 2 and with 3–4 mismatches is statistically almost significant ($P < 0.05$) the cell densities being [illegible]09 ± 463 (N = 15) and 658 ± 235 cells/mm² (N = 7)

This analysis thus shows that regardless of whether the patient s cornea was or was not vascularized the average endothelial cell density of the transplant among compatible pairs (means 0–1 (2) mismatches) was clearly above the mean cell density for the whole series Also the mean cell densities in the groups with 2 mismatches were close to the mean for the whole series

ABO compatibility and graft endothelial cell density

Table V shows the cell numbers in different ABO-compatibilities Although the ABO-compatible group had 18% more endothelial cells than the ABO-incom

Table V
Effect of ABO blood group compatibility of the endothelial cell densities of the transplants

Blood group compatibility	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells/mm²)
(ABO) Compatible	69	(66) 50 0	42 3	12 8 ± 48
(ABO) Incompatible	11	(11) 48 5	33 1	1033 ± 26
Untyped donors	22	(19) 46 3	40 0	1011 ± 30

patible group the difference was not statistically significant When the ABO compatible pairs were compared with all the others the cell densities of the two groups differed almost significantly ($P < 0.05$)

Effect of rejection episodes on graft endothelial cell density (Table VI)

Eight patients in this series had had a reversible rejection episode but the cornea had cleared sufficiently for photography of the endothelium In this small series of rejected transplants the mean endothelial cell density was 945 ± 254 cells mm² and in the grafts with an uneventful postoperative course (N = 94) the cell density was 1188 ± 188 cells/mm² Thus although there was 20% fewer endothelial cells mm² in rejected grafts than in normal ones the difference in cell density was not statistically significant

Table VI
Effect of reversible rejections on the endothelial cell densities of the transplants

	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells mm²)
Rejection	8	45 1	33 3	945 ± 254
No rejection	94	(88) 49 4	41 6	1188 ± 188
Total	102	(96) 49 1	40 8	1163 ± 48

servation period after rejection

n one patient with keratoconus the endothelial cell density w as cle irly affected by rejection Rejection (Fig 1) occurred three years after tr insplant ition and 2 months after the rejection episode the cell density w is 112 cells/mm² which w is included in the results for the series of 102 patients (Fig 2 i) Two ye irs l iter the *mean cell density had decreased to 550 cells/mm² (Fig 2b) The l iter photogr iphs* was just a routine check up and not regarded is adequ ite for ch inging of the statistics In the present study the average follow up period after rejection v is 2 years

Discussion

In Bourne & Kaufman s (1976b) series of untyped donors the me in endotheli il cell density correspond with the result of untyped pairs in this study (959 ± 313 cells/mm²) but better cell densities were found in more compatible pairs

The negative correlation between the incompatibility of donor recipient pairs and the endothelial cell density of the graft tells us that the best possible result will be obtained with 0–1 mismatch pairs Endothelial cell loss is consider ible in p urs involving 3–4 mismatches or untyped donors Corneal antigens result in some kind of subclinical rejection episodes which probably occur to some degree in every incompatible transplant According to Khodadoust & Silverstein (19) all corneal layers may be rejected But for the maintenance of clarity and deturgescence in the graft the most important layer is probably the endothelium The less comp itil le the



F g 1

Fluorescein angiographs of the cornea *A rejection episode with e endo ve ilation* in the upper part f the gr ft The *mer gr ph was taken b e th*

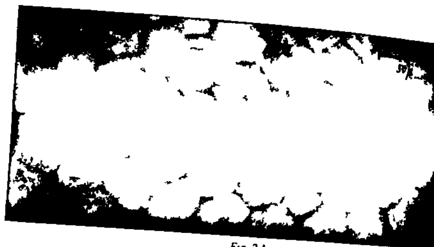


Fig 2A

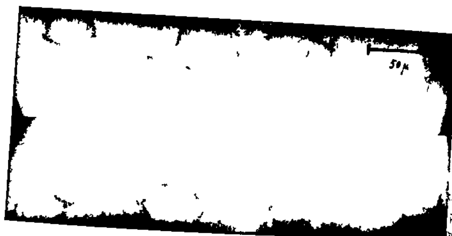


Fig 2B

A Central corneal endothelium of the same cornea as in Fig 1 seen 9 months after the rejection The mean cell density is 1125 cell/mm² Two different fields are shown together
B The same central corneal endothelium as in Fig 2A seen 2 years after the rejection The mean cell density is [illegible]0 cells/mm²

transplant the greater the endothelial cell loss due to subclinical rejection Conclusive evidence about the effect of latent rejection on endothelial cell dens[ity] will probably require long follow up periods

In our earlier study (Vannas et al 1976) we noticed the clinical importance of HLA typing in corneal grafting In that series rejection episodes were seen to occur more often in the untyped and the less compatible donor recipient pairs Th[e] present study shows that a correlation exists between endothelial cell loss a[n]d degree of HLA incompatibility

There are of course other factors which may affect the endothelial cell density of the graft e g donor age cadaver time and postoperative period (Ruusuvaara in press) One of the most important is donor age (Bourne & Kaufman 1976b Sato 19 Ruusuvaara in press) As Table II shows between the groups based on the number of mismatches the differences in mean donor age were not statistically significant and cannot account for the difference in endothelial cell density between the different groups The mean age of the recipients was also almost identical in the different groups The postoperative periods were longer (about five years) for the more incompatible pairs (3–4 mismatches untyped) than for the compatible pairs (rather less than two years) Although this will tend to impair the results for the incompatible pairs it does not explain the whole difference still longer follow up for compatible grafts are needed to show how much better the cell survival will be in the long run

In regard to the relation between the diagnosis of the recipient and the endothelial cell density of the transplant it was found that the cell density of the graft was lower in the patients with keratoconus than in the other patients Another point that emerged was the low endothelial cell densities in the patients with other corneal dystrophies The patients with keratoconus or other dystrophies had fewer cells in their corneal grafts than the other patients and this poorer result was independent of vascularization But as Table IIa shows in the patients with keratoconus a good HLA match seems to be nearly as important as in the patients with other diseases in whom the cell density of the graft clearly decreased with increasing incompatibility So it seems that regardless of diagnosis tissue compatibility is of great value (Chandler & Kaufman 1974)

The importance of ABO compatibility for the clinical results of corneal transplantation is controversial In previous studies (Meyer 1966 Alberth 1968) the only precaution taken to avoid immune reactions was the determination of ABO compatibility HLA antigens are present in the human cornea (Ehlers & Ahrons 1971) and so HLA compatibility may also be an important factor in immune reactions against corneal grafts

In a follow up study of ABO-compatible transplantations Ehlers & Kissmeyer Nielsen (1973) found that clear grafts were significantly more frequent among the more HLA-compatible pairs But in their series pairs with only 0–1 or 2 mismatches were uncommon because no attempt had been made to select good matches In fact their series was randomly selected with respect to histocompatibility matches

In this series the endothelial cell density in the ABO-compatible donor recipient pairs was higher than in the small number of ABO incompatible pairs However the difference was not statistically significant But when the ABO-compatible patients are compared with the ABO incompatible and untyped patients the graft endothelial cell densities show a statistically significant difference

Vascularization of the recipient cornea is believed on good grounds to jeopardize the success of keratoplasty. Interestingly, in a series of 585 keratoplasty patients Capella et al. (1972) found that vascularization of the cornea seemed to play a relatively small role in the long term clinical prognosis. They showed that even eyes with severe vascularization may do well after keratoplasty and the prognosis for such eyes does not differ greatly from that for eyes without corneal vascularization. The present study shows that the endothelial cells of the graft survive quite well in a vascularized bed. One explanation is that half of the patients with vascularized corneas belonged to the 0–1 (2) mismatches group. Comparison of the effect of tissue incompatibility on the graft endothelial cell density separately in the patients with vascularized and non vascularized corneas (Table IV) showed that in both of these categories the endothelial cell density was highest in the group with only 0–1 (2) mismatches. A compatible cornea thus seems conducive to a good result whether the cornea is vascularized or not.

In spite of anti-inflammatory therapy, eight patients had had reversible clinical rejection episodes. The average cell density in these patients at follow up was about 20% less than in the patients without rejection. After reversible rejection some patients had very low cell densities in the central corneal endothelium. In one patient with keratoconus vascularization appeared in the upper part of the transplant 3 years postoperatively, and the rejection seemed to have a distinct effect on the central corneal endothelial cell density (Fig. 2a and b).

This indicates that rejection does affect central cell density, but the change is visible only after a considerable period, perhaps even years. The type of rejection and the corneal layer affected in the rejection may also be decisive.

Although the difference between cell densities in patients with and without rejection is not statistically significant, it seems that rejection episodes may decrease the central endothelial cell density to some degree, though in most cases rejection is limited to the peripheral part of the transplant. Bourne et al. and Sato had previously reported similar effects of rejection on the endothelium. So it is very important to prevent a rejection episode by anti-inflammatory therapy as early as possible.

In this series it seems that pairs with 0–1 HLA mismatch have the best chances of endothelial survival. The results also suggest that untyped pairs or pairs with 3–4 mismatches suffer from subclinical rejection, with a subsequent decrease in endothelial cell density to levels that are clearly lower in the mismatched and untyped groups than in the other groups.

References

lberth B (1968) *Surgical treatment of caustic injuries of the eye* Akademiai Kidao Budapest.
llansmith M R Fine M & Payne R. (1974) Histocompatibility typing and corneal tran plantation *Trans Amer Acad. Ophthal Otolaryng* 78 445–460
mos D D Bashir H Boyle W MacQueen M & Tilikainen A. (1969) A simple microcytotoxicity test. *Tran. plantation* 7 220–222
atchelor J R (1976) HLA matching and corneal grafting *Lancet I* 551–554
ourne W M & Kaufman H E. (1976a) Specular microscopy of human corneal endothelium in vivo *Amer J Ophthal 81* 319–323
ourne W M & Kaufman H E (1976b) The endothelium of clear corneal transplants *Arch Ophthal. 94* 1730–1732
apella J A Kaufman H E & Polack F M (1972) Prognosis of keratoplasty in phakic and aphakic patients and use of cryopreserved donor tissue *Trans Amer Acad Ophthal Otolaryng* 6 1265–1285
apella J A. Kaufman H E & Robbins J E (1965) Preservation of viable corneal tissue *Cryobiology* 2 116–121
handler J W & Kaufman H E. (1974) Graft reactions after keratoplasty for keratoconus *Amer J Ophthal.* 77 543–547
hlers N & Ahrons S (1971) Corneal transplantation and histocompatibility *Acta ophthal. (Kbh.)* 49 513–527
hlers N & Kissmeyer Nielsen F (1973) Influence of histocompatibility on the fate of the corneal transplant. In *Corneal graft failure* Ciba Foundation Symposium 15 (n s) pp 307–319 Associated Scientific Publishers Amsterdam
ibbs D C. Batchelor J R. & Casey T A (1973) Influence of HLA compatibility on the fate of corneal grafts In *Corneal garft failure* Ciba Foundation Symposium 15 (n s) pp 293–306 Associated Scientific Publishers Amsterdam
ones B R. (1973) Present knowledge and problems of corneal graft failure In *Corneal graft failure* Ciba Foundation Symposium 15 (n s) pp 349–354 Associated Scientific Publishers, Amsterdam
hodadoust A. A. & Silverstein A M (1969) Transplantation and rejection of individual cell layers of the cornea. *Invest. Ophthal.* 8 180–195
aing R. A., Sandstrom M M & Leibowitz H M (1975) In vivo photomicrography of the corneal endothelium *Arch. Ophthal (Chicago)* 93 143–145
Meier H J (1966) Klinische und experimentelle Untersuchungen zur Bedeutung von Isoantikorpern bei Keratoplastik. Habilitationsschrift Gottingen Cited by Boke W (1968) Immunopathologie des Auges *Bibl. Ophthal* 78 61–96
Polack F M (19 3) Clinical and pathological aspects of the corneal graft reaction *Trans Amer Acad. Ophthal. Otolaryng* 77 418–422
Ruusuvaara P (1979) Effects of corneal preservation donor age cada er time and postoperative period and the graft endothelium Specular microscopic study *Acta ophthal (Kbh.)* 57 868–881
Sato T (19 8) Studies on the endothelium of the corneal graft *Jap J Ophthal.* 22 114–126
Vannas S Karjalainen K Ruusuvaara P & Tiilikainen A (19 6) HLA-compatible donor cornea for prevention of allograft reaction *Albrecht v Graefes Arch Kl n exp Ophthal* 198 1 992
Vannas S Tiilikainen A Vannas A & Karjalainen K (1978) HLA B12 and HLA B27 antigens and susceptibility to the corneal allograft reaction *Acta ophthal. (Kbh.)* 56 689–696

Author's address

Pekka Ruusuvaara M D The University Eye Clinic
University of Helsinki SF-00190 Helsinki 29 Finland

Department of Ophthalmology (Head N Ehlers) and the Blood Bank and Tissue Typing Laboratory (Head F Kissmeyer Nielsen) Århus Kommunehospital University of Aarhus Denmark

HLA TYPES IN CORNEAL DISEASES

BY

LARS DAMGAARD JENSEN NIELS EHLERS and
FLEMMING KISSMEYER NIELSEN

Evaluation of the results of HLA typing of 187 patients grouped into herpetic keratitis (37) non herpetic keratitis (43) keratoconus (49) endothelial dystrophy (23) stromal dystrophy (13) lues (5) and injuries (24) failed to show convincing deviations in any of the groups from a normal control series (900 persons) Yet as for the herpes group a rise in B5 must strongly be suspected. The data are presented and possible implications are discussed

Key words HLA – keratoconus – keratitis – Fuchs endothelial dystrophy stromal dystrophy

Within medicine interest is increasingly being focused on HLA tissue typing Th outcome of kidney grafting depends on the degree of HLA compatibility betwee donor and recipient and this applies also for corneal grafting (Ehlers & Kissmeye Nielsen 1973) Tissue typing has shed light on hereditary aetiologic and prognost aspects of many diseases Tissue typing is rather expensive and in order to avo superfluous repetitons of positive as well as negative studies Svejgaard et al. ha taken the praiseworthy initiative of establishing an HLA and Disease Registry Th third report from this registry is to be published in the near future (Ryder et al. press)

In an earlier paper (Damgaard Jensen & Kissmeyer Nielsen 1978) the results HLA typing in open angle glaucoma were summarized No deviations from t normal population were found

Zimmermann et al (1977) suggest but do not prove a rise in HLA B5 in patie with recurrent corneal herpes simplex infection According to Yannas et al (197

Received March 28 1979

HLA is of prognostic significance for the result of corneal grafting in keratoconus as the occurrence of B12 and B27 is significantly higher in the group of graft failure.

Since 1968 we have HLA typed corneal donors and recipients with the purpose of gaining information about the importance of HLA compatibility for the fate of the graft. These results have been published in 1971 and 1973 and now the publication of an analysis of a series of about 200 full-house matched cases is in preparation. The number of cases in the different diagnostic groups are now so great that it allows an analysis of the frequency of the HLA antigens in the diagnostic groups compared with the findings in a large group of normal blood donors.

Material and Methods

... patients comprising 37 cases of herpetic keratitis, 43 cases of non-herpetic keratitis, 42 cases of keratoconus, 23 cases of Fuchs' endothelial dystrophy, 24 cases of injuries, 13 cases of stromal dystrophy and 5 cases of syphilitic corneal changes were compared to a large series of normal blood donors (2960, slightly fewer for some antigens). The HLA types were determined by the cytotoxicity test according to Kissmeyer-Nielsen & Kjerbye (1967).

The calculations were undertaken by electronic computer and chi-square test with Yates correction was used, except where the numbers were too small in which case Fisher's exact test was applied.

Results

The results appear from Table I, which shows the 2P values at and below the ... level. The rest of the results will be delivered on application to the HLA and Disease Registry, address: A. Svejgaard, M.D., Tissue Typing Laboratory BL 7911, Rigshospitalet, Blegdamsvej 9, DK 2100 Copenhagen Ø, Denmark. It appears that the distribution of 2P values does not deviate from what might be expected. Correction a.m. Grumet (multiplication of the 2P value by the number of antigens investigated (= 31) (Grumet et al. 1971)) eliminates any trend of significance.

Discussion

... calculations (31 antigens and 7 diagnostic groups) were made, and the 2P values were about the 1% level in only 3 cases (2 in the keratoconus group and one in the Fuchs dystrophy group). This was what one might have anticipated, and

Table I

Deviations (compared to a healthy control group) of HLA types of various diagnostic groups of corneal diseases

Diagnosis	HLA type	No. of patients	No. of positive patients ( ) = %	No. of controls	No. of positive controls ( ) = %	2P
Herpetic keratitis	A2	37	14 (37.8)	[illegible]	[illegible]	0.063
	B5	37	8 (21.6)	[illegible]	[illegible] (10.4)	0.03
	Cw3	28	5 (17.8)	[illegible]	820 (34.8)	0.061
Non-herpetic keratitis		no deviation				
Keratoconus	B7	42	5 (11.9)	[illegible]	833 (29.1)	0.014
	B15	40	14 (34.9)	[illegible]	542 (18.9)	0.010
Fuchs endothelial dystrophy	B12	23	11 (47.8)	[illegible]	746 ([illegible]6.1)	[illegible]
Stromal dystrophy	A28	12	4 (33.3)	2856	241 (8.4)	0.056
	B7	13	0 (0.0)	[illegible]	833 (29.1)	0.058
	B18	12	4 (33.3)	2861	217 ([illegible])	[illegible]
Lues		no deviation				
Injuries		No deviation				

the primary conclusion is accordingly that the results are entirely negative. However, a priori one could have expected the frequency of the antigens to deviate from the normal population in case of keratoconus (heredity, association to atopic diseases), herpetic keratitis (possibly an immunological defect), Fuchs endothelial dystrophy (defects in the fibrinolytic and the complement system (Bramsen & Stenbjerg, 1979)) and possibly in stromal dystrophies (heredity). These expectations are to some degree fulfilled as the borderline significances were in the keratoconus and the endothelial dystrophy groups. Futher investigations are needed to confirm or invalidate the suggestions. As regards herpetic keratitis an elevated frequency of B5 has been reported ($\chi^2 = 7.13$, 2P about 0.01) (Zimmermann et al. 1977). Summation of these results with those of the present investigation show a rise in B5 with a chi square of 14.12, corresponding to a 2P value ≤ 0.001. Provided that Zimmermann et al.'s finding (1977) is regarded as a preliminary result to be invalidated or confirmed by another similar investigation (such as the present one)

seems justified to regard a rise of B7 in herpetic keratitis as being rather strongly indicated (in that case correction a nt Crumet is unnecessary and the result of the one-sided P test is $P = 0.036$). This stresses the need to avoid attaching importance to the results of one single investigation whether they are negative or positive.

Acknowledgments

Chief laboratory worker Lisbeth Wolthers, The Tissue Typing Laboratory, Århus Kommunehospital, University of Aarhus, Denmark, is thanked for her invaluable assistance. Further, Arne A. Svejgaard, M.D., Tissue Typing Laboratory, Rigshospitalet, Copenhagen, Denmark, is thanked for his skilful advice.

References

Bramsen T. & Stenbjerg S. (1979) Variations in serum antifibrinolytic activity following eye operations. Acta ophthal. (Kbh.) 57, 470–476.

Damgaard Jensen L. Kissmeyer Nielsen F. (1978) HLA histocompatibility antigens in ... angle glaucoma. Acta ophthal. (Kbh.) 56, 384–388.

Ehlers N. & Kissmeyer Nielsen F. (1973) Influence of histocompatibility ... In: Corneal Graft Failure. Ciba Foundation Symposium I...

Grumet F. C., Coukell A., Bodmer J. G. & McDevitt H. O. (1971) Histocompatibility ... associated with systemic lupus erythematosis. New Engl. J. Med. 285, 193–1...

Ryder L. P., Andersen E. & Svejgaard A. (1979) HLA and disease. Third report ... HLA and disease registry. Munksgaard, Copenhagen. In press.

Kissmeyer Nielsen F. & Kjerbye K. E. (1967) In: Histocompatibility ... Mattiuz P. L. & Tosi R. M. (eds.), pp. 381–383. Munksgaard, Copenhagen.

Sarks W. J., Taylor H. R., Bias W. B. & Maumenee A. E. (1978) Histocompatibility ... and keratoplasty. Amer. J. Ophthal. 86, 592–604.

Vannas S., Vannas A. & Kajalainen K. (1977) HLA Antigens ... Keratoconus. Klin. Mbl. Augenheilk. 170, 391–396.

Zimmermann T. J., McNeil J. I., Richman A., Kaufman H. F. & Waltman S. R. (1...) HLA types and recurrent corneal herpes simplex infection. ... Ophthal. ...

Author's address:
Niels Ehlers, Department of Ophthalmology,
Århus Kommunehospital, DK 8000 Århus C, Denmark.

Department of Ophthalmology (Head: N. Ehlers), University of Aarhus, Århus, Denmark

NON CONTACT SPECULAR MICROSCOPY OF HUMAN CORNEAL ENDOTHELIUM

BY

THOMAS OLSEN

The non-contact specular microscope enables a large area of the corneal endothelium to be examined and photographed without any risk of traumatizing the anterior surface of cornea. This non-contact technique is described in detail. Standard deviation of the relative difference between two independent estimates of central endothelial density from 94 eyes was 3.7%. Standard deviation of the relative difference between counts from left and right eyes from 57 subjects was 4.7%. A significant negative correlation between age and endothelial cell density was found ($r = -0.59$, $P < 0.001$, $n = 76$). The interindividual variation in cell densities was found to be larger in the old age group, indicating that cell loss during aging varies among different individuals.

Key words: specular microscopy – non-contact – cornea – endothelium – age

The technique of visualizing the endothelium by specular illumination with th[e] slit lamp was originally described by Vogt (1920). The magnification available wi[th] ordinary biomicroscopes is however not sufficient for a detailed study of the cellul[ar] morphology of the endothelium. When Maurice introduced the specular micr[o]scope these problems were largely overcome (Maurice 1968) so that it becam[e] possible to examine and photograph the human corneal endothelium at hig[h] magnification *in vivo* (Laing et al. 1975, Bourne & Kaufman 1976, Sturrock [&] Sherrard 1978). This specular microscopy technique implies applanation of corne[a] with a dipping cone mounted on the outside of a microscope objective. Th[e] applanation is of help in keeping the endothelial cells in focus and allows a hig[h] magnification (about × 200).

Received April 17, 1979

This contact technique has several disadvantages however The applanation requires the use of a topical anaesthetic and the physical contact with the patients eye carries a risk of traumatizing the anterior surface of cornea This risk is even higher in the case of a diseased cornea where opacity of the cornea may hinder or prolong the examination Furthermore the area of endothelium observed at a time with the most clinical specular microscopes is rather small making deductions as to the state of the whole cornea difficult

Only a few attempts have been made to perform high magnification specular microscopy without contact with the cornea Using a macrophotographing system Brown demonstrated that the endothelium can be photographed using a non contact technique (Brown 1970 Bron & Brown 1974) Photographs obtained with the slit lamp have also been reported (Laule et al 1978) The endothelial photos obtained were clearly not as good as those obtained by contact specular microscopy ad modum Maurice Recently a more successful attempt has been reported by Holm (1978) The high magnification in his technique was simply achieved by a horizontally mounted specially fitted microscope supplied with an objective with a long working distance (5 0 mm) With slight modifications this technique has been adopted by us

The purpose of the present paper was to evaluate the clinical usefulnes f the non-contact specular microscope technique to demonstrate the reproducibility of sampling of endothelial cell densities with this technique and to presen data on normal intra and interindividual variations in endothelial cell densitie pti principles for estimation of endothelial cell density with this tech que ha been described (Olsen 1979)

Methods

The non-contact specular microscope (Fig 1) was made by Preisler Instrument Malmo Sweden after suggestions by Dr Olle Holm University of Lund Sweeden (Holm 19 8) It consists of a microscope tube (A) mounted with a microscope objective (B) with a long working distance A reflex camera (C) is mounted directly on the tube The ocular of the camera thus functions as ocular of the microscope A focusing thread (D) connects the microscope set up to a special bracket (E) designed to fit a Zeiss photo slit lamp

In the present microscope set up an objective with an extra long working distance (1 mm) was used (Leitz 25 × n a 0 22) This long working distance permits photographs to be taken without physical contact with the patient s eye and furthermore a small angle can be obtained between incident slit illumination and reflected light The angle used in the present study was fixed at 46 by mechanically fixing the slit lamp and the microscope to each other This small angle duces a constant error in the horizontal dimension which can be corrected for when endothelial density is estimated (Olsen 1979) The slit illumination is provided by the photo

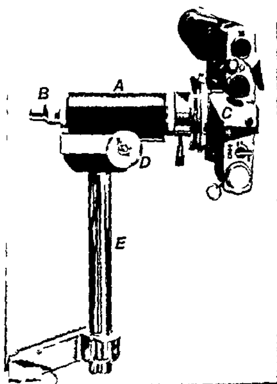


Fig 1

The non contact specular microscope A microscope tube B objective with a long working distance C reflex camera D focusing thread F bracket designed to fit a photo slit lamp

slit lamp (Zeiss) When photographs are taken both the reflex from the endothelium and the reflex from the anterior surface at the cornea are seen Due to its large intensity the later reflex tends to overflow the reflex from the endothelium an effect which is even more disturbing when the film is exposed In order to increase the area illuminated on the endothelium relative to the area on the epithelium the ordinary lamp head which gave a narrow beam of light was replaced by a lamp head which gave a more converging slit illumination A suitable plus lens was inserted between the light source and the plane of focus and adjusted so that the slit light was focused on the epithelium when photographs of the endothelium were obtained In this way an increased area on the endothelium can be illuminated

In order to obtain photographs from a defined and reproducible area on the central cornea a small fixation light was placed between the slit lamp and the microscope (Fig 1) The long working distance of the objective enables the eye under examination to look at the fixation light which is placed on the angle bisector between incident and reflected light. If the slit lamp the specular microscope and fixation light are fixed mechanically to each other

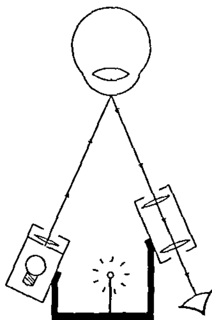


Fig. 2

a fixation light is placed on the angle bisector between incident and reflected [illegible]
area on the cornea is defined

[illegible]crete area on the endothelium is defined if the eye is rotated it doe[illegible]
[illegible]t, and if it is moved up- down- back or forwards the reflex is not seen [illegible]
[illegible]rosc pe In case of impaired vision of the eye under examination [illegible]
[illegible]ntralateral eye another fixation light was used to direct the gaze of the contralateral eye
Photomicrographs were taken using a flashing power suppl (Siemens) [illegible]
[illegible] and white films were used (Agfa Pan 25 24 × 36 mm 15 DIN) [illegible]
[illegible]ndary enlargement (18 × 24 cm) of the negative All positives were made at exactly the
[illegible] magnification using scale proof plastic paper (Kodak F 4 18 × 24 cm) Final magni[illegible]
[illegible]ation was determined by photographing a Burger Turk blood cell counting chamber
A total of about four to eight photographs were taken of each eye On the basis of the
[illegible] photographic clarity two photomicrographs were selected on which a total of three
[illegible] (1 × 3 3 cm each) were drawn The area of the frame corresponded to 0 01[illegible]9 mm²
[illegible] cornea. The number of cells within the frames was counted following the counting
[illegible]ciple described by Gundersen (Sperling & Gundersen 19 8) which allows unbiased
[illegible]mates to be drawn from small samples The mean cell count was taken as an estimate of
[illegible] density

The normal subjects included in this study comprised medical students members of the
[illegible]cal staff and patients hospitalized due to squint or senile lens opacities Eyes with any
[illegible] of trauma or disease past or present other than squint or senile cataract were
[illegible]cluded.

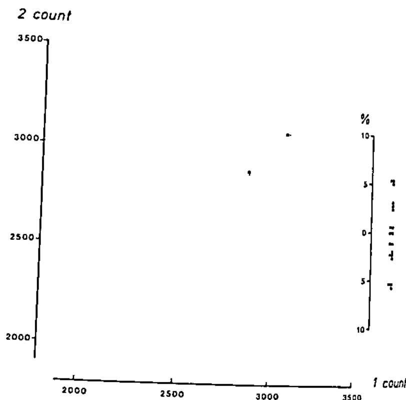


Fig 3
Reproducibility of counting technique demonstrated by comparing two estimates of central endothelial cell density obtained with an interval of 3 to 14 days in 24 normal eyes. Dotted line indicates the graph y = x. Insert shows the relative difference

$$\left(\frac{2 \text{ count} - 1 \text{ count}}{1 \text{ count}} \times 100\% \right)$$

SD of relative difference is 3.7%

Results

Reproducibility of cell counting

Twenty four normal eyes (age range 27–53 years) were photographed and rephotographed after an interval of 3 to 14 days. Endothelial photographs were obtained using the fixation light arrangement depicted in Fig. 2. Estimates of endothelial cell density were obtained in a 'blind' fashion by mixing all photographs prior to counting. The result is shown in Fig. 3. Endothelial cell density ranged from 2200–3200. The standard deviation of the difference between first and second cell count was 3.7%. To see how effective the fixation light arrangement worked some photographs were later scrutinized in order to see if it was possible



F g 4

The same end thelial area repht tographed after one weeks interval For help f nerta three cells are identified by asterixes Bar = 100 µm

identify the same area Due to the uniformity of the endothelium this i gener very difficult. In one instance however it was actually possible to identify the sa cells one week after first examination (Fig 4) The spatial arrangement of the cell seen to be unchanged

Corre ation with age and difference between right and left eye

In Fig. 5 the mean cell count of subjects versus age is plotted When both eyes were photographed a mean of the two counts was employed A negative relation hip appears (r = −0 59 $P < 0.001$) A characteristic feature is the large variation seen in all age intervals which however is more pronounced in the old age group than in the young age group If the counts in the age group of 70–80 years is compared to the group of 20–30 years a significant smaller variation is seen in the younger age group (Table I) No differences were observed between cell counts from men or women in any of the age groups Examples of the variation in cell density in normal endothelia is shown in Fig 6

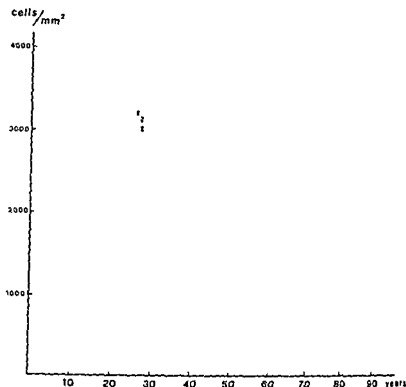


Fig 5
Correl ation between age and cell count in 16 subjects (r = −0.59 P < 0.001)

A high concordance between counts from right and left eyes was found (Fig 7), mostly within the counting error (compare Fig 3). Counts from left eyes were found to be slightly (1 8%) lower than counts from right eyes (P < 0 01). Standard deviation of the difference between right and left count was 4 7%. The numerical relative difference between right and left eye was found to increase significantly with increasing age (r = 0 34, P < 0 05).

Table I
Inter individual variation in estimates of endothelial cell density in a young and an old group of normal subjects

Age	n	Cells mm² $\bar{x}$	Cells mm² SD	F test
20 − 0	20	3032	248	F = 9 58
70 − 80	16	2509	398	(P < 0 05)

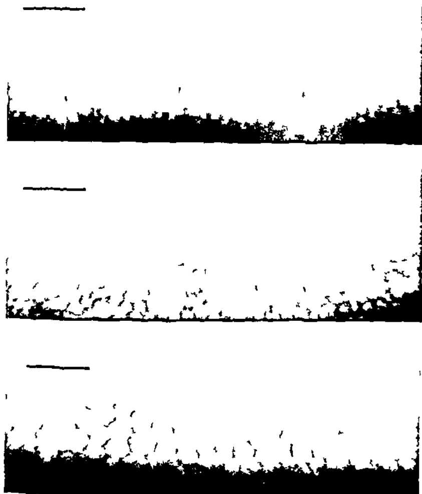


Fig 6

Normal human corneal endothelium Top male 6 years of age endothelial cell density 3560 cells mm² Center female 76 years of age endothelial cell density 2960 cells mm² Bottom male 70 years of age endothelial cell density 1260 cells mm² Bar indicates 100 µm

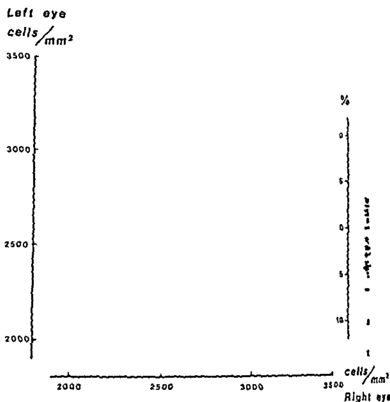


Fig 7

Correlation between estimates of central endothelial cell density from right and left eyes subjects Dotted line indicate the graph of $y = x$ Inseert shows the relative difference between the two eyes

$$\left(\frac{\text{left eye count} - \text{right eye count}}{\text{right eye count}} \times 100\%\right)$$

Mean relative difference is −1 81% which is significantly different from zero ($P < 0.01$ basis of the Student distribution) SD of relative difference is 4

Endothelial morphology

The non contact specular microscope is a sensitive guttae det tion of the endothelial cell layer from the plane is in the specu as black areas (Fig 8) That these black areas really are gu cell free areas is proved by slightly altering the focusing whereby the endothelium lining the guttae can be seen often seen in the old age group but isolated minc observed in younger persons (20−30 years of age)



Fig 8

Endothelial guttae as seen with the specular microscope Top male 63 years old few guttae visible with the slit lamp Bottom female 79 years old numerous guttae visible with the lamp Bar = 100 μm

Discussion

This study has shown the non-contact specular microscope to be a reliable and useful tool in investigating the human endothelium Due to the lower magnification and the greater angle of observation it provides a greater view of the endothelium than do most contact specular microscopes (Bourne & Kaufman 1976 Laing et al 1976 Sturrock & Sherrard 1978) This reduces the error when estimates of endothelial cell density are obtained Furthermore the non-contact technique enables a *highly defined area of the endothelium to be photographed (Fig 2) which increases the accuracy and reproducibility of the method even more*

The decrease in cell density with age has been found by several authors (Laing et al 1976 Bourne & Kaufman 1976 Laule et al 1978 Sturrock & Sherrard 1978) although the magnitude of this decrease differs considerably among different investigators In a study of necropsy eyes Irvine & Irvine (1953) found no age dependent variations in cell density On the other hand a very large dependence was reported by Laule er al (1978) The non-contact technique used by the latter

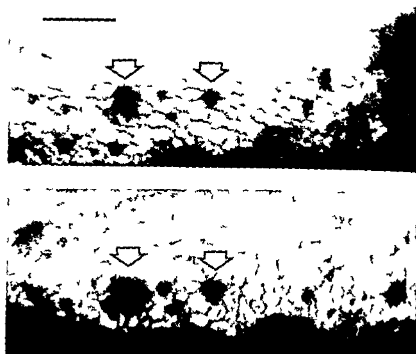


Fig 9
The size of guttae seen in the specular reflex is highly dependent on the angle of observation and the focusing depth which were altered slightly between these two photographs
Bar = 100 µm

necessitates information regarding the angle of observation in order to correct for distorted dimensions on the photomicrographs (Olsen 1979) This factor was neglected in the study by Laule et al Sturrock & Sherrard (1978) found an age dependent change in cell density which seems similar to that of the present paper These authors also demonstrated the reproducibility of their counting technique

The great variation in cell density among different individuals need to be stressed The present study also shows this variation to be age-dependent so that the smallest variation is seen in the young age groups This together with the weak correlation between age and cell density means that the highest cell counts in the old age group are not very different from the counts in the group of 20–30 years This indicates the cell loss during aging to vary from one individual to another and to actually be rather small in some persons

Whereas a large variation exists between cell densities from different individuals the small variation between left and right eye from the same individual is

markable (Fig 7) Mostly this difference is within the counting error but in some ses a significant difference is found The age dependence of this difference found the present study was also found by Laing et al (1976) The high concordance tween left and right eye offers an opportunity of retrospectively assessing dothelial damage following unilateral trauma or disease

The specular microscope often revealed endothelial guttae which were not seen the optical section of the ordinary slit lamp In many instances these guttae were ry small i e the area with the absent reflex comprised one or two cells width but e size was highly dependent on the focusing depth and the angle of observation. ue to the frequent occurrence in otherwise normal corneas minor guttae were not ken as cause of exclusion from the normal corneas in the present study Corneas th guttae which were readily seen in the slit lamp were excluded

In conclusion it can be said that non-contact specular microscopy is a reliable and producible technique for examination of human endothelium It is safe and uses no discomfort to the subject due to the non touch technique Furthermore mbined with a special fixation light arrangement it enables a defined area on the dothelium to be observed after periods of time The clinical and experimental fulness of the non-contact specular microscope is thus fully comparible to and some instances even greater than that of ordinary contact specular microscopes

Acknowledgements

is work was supported by grants from Fonden til Lægevidenskabens Fremme The hnical assistance of mrs Anette Poulsen is gratefully acknowledged

References

urne W M & Kaufman H E (19 6) Specular microscopy of human corneal endothelium *vivo Amer J Ophthal 81* 319–323

on A. J & Brown N A P (1974) Endothelium of the corneal graft. *Trans Ophthal Soc K 94* 863–873

own N (1970) Macrophotography of the anterior segment of the eye *Brit J Ophthal 54* 97–701

lm O (1978) High magnification photography of the anterior segment of the human eye Transactions of the Swedish Ophthalmological Society 1977 *Acta ophthal (Kbh.) 56* 4 5–4 6

ne A R & Irvine A R *(1953) Variations in normal human corneal endothelium Amer J Ophthal 36* 1279–1285

ing R A Sandstrom M M Leibowitz H M (1975) *In vivo* photomicrography of the corneal endothelium *Arch Ophthal (Chicago) 93* 143 145

Laing R A Sandstrom M M Berrospi A R Leibowitz H M (1976) Changes in the corneal endothelium as a function of age Exp Eye Res 22 587–594
Laule A Cable M K Hoffman C E & Hanna C (1978) Endothelial cell population changes of human cornea during life Arch Ophthal (Chicago) 96 2031–2035
Maurice D M (1968) Cellular membrane activity in the corneal endothelium of the intact eye Experientia 24 1094–1095
Olsen T (1979) Optical principles for estimation of endothelial cell density with the non contact specular microscope Acta ophthal (Kbh) In press
Sperling S & Gundersen H J G (1978) The precision of unbiased estimates of numerical density of endothelial cells in donor corneas Acta ophthal (Kbh) 56 793–802
Sturrock G D Sherrard E S & Rice N S C (1978) Specular microscopy of the corneal endothelium Brit J Ophthal 62 809–814
Vogt A (1920) Die Sichtbarkeit des lebenden Hornhautendothels Ein Beitrag zur Methodik der Spaltlampen mikroskopie Graefes Arch Ophthal 101 123–127

Author's address

Thomas Olsen Department of Ophthalmology Århus Kommunehospital
DK 8000 Århus C Denmark

Department of Ophthalmology[1] (Head: M. N. Luxenberg) and
Department of Physiology[2] (Head: Robert Little)
Medical College of Georgia, Augusta, Georgia, U.S.A.

CORNEAL ENDOTHELIAL PERMEABILITY FOLLOWING STORAGE IN MOIST CHAMBER AND MK MEDIUM

BY

DAVID S. HULL[1], KEITH GREEN[1,2] and KAREN BOWMAN[1]

Rabbit corneal endothelial permeability to sucrose and dextran was determined after storage in moist chamber or MK medium. When compared to fresh rabbit corneas there was no statistically significant difference in endothelial permeability to these two solutes after both types of storage for periods up to ten days.

Key words: rabbit cornea endothelium – permeability – tissue preservation – tissue storage – corneal preservation

Experimental and clinical work has demonstrated the efficacy of preserving corneas in intermediate term preservative MK medium TC 199 plus 5% dextran (McCarey & Kaufman 1974; Bigar et al. 1975; Stark et al. 1975). Van Horn et al. (1975), however, have shown by both trypan blue staining and incubation prior to fixation for scanning and transmission electron microscopy that a significant number of cat endothelial cells are non viable after storage for periods longer than four days in MK medium. It was the purpose of this investigation to compare the endothelial permeability to non transported non-electrolytes of rabbit corneas stored in MK medium and moist chambers for period up to ten days and thereby assess and compare endothelial barrier function following storage by the two techniques.

Received March 22, 1979.

Materials and Methods

Adult albino rabbits were sacrificed with an overdose of sodium pentobarbital and the eyes were enucleated Either the entire eye was stored in a moist chamber at 4°C, or a cornea with a scleral rim was removed and stored in MK medium at 4 C Corneas stored for periods of four and ten days by each technique were then atraumatically mounted in a perfusion chamber at 37 C and the endothelial surface and epithelial surface bathed in a modified Krebs Ringer bicarbonate (KRB) solution (Green & Green 1969) with adenosine (134 mg/l) and reduced glutathione (92 mg/l) (Dickstein & Maurice 1979) In addition the bathing solution contained either sucrose or dextran at concentrations of 10 mM or 1 0 mM respectively Corneas as prepared above or from freshly enucleated eyes were bathed for ten min in either the KRB sucrose or KRB dextran solution in order to achieve osmotic equilibrium. The procedure was followed to eliminate the potential influence of bulk osmotic water movement influencing solute movement into the cornea Mishima & Hedbys (1967) demonstrated that over 95% of thickness change (and hence net fluid movement) was complete within 10 min after exposure to similar osmotic solutions Thereafter the solution perfusing the endothelial surface was removed and replaced by one containing all of the constituents of the initial bathing solution plus trace amounts of ^{14}C sucrose (8 1 mCi/mM) or ^{14}C dextran (1 mCi/mM) Corneas were then bathed for an additional 30 min after addition of the labelled compound to the endothelial perfusing chamber After 30 min an aliquot from the endothelial perfusing chamber was sampled and the corneas removed Corneas were washed briefly in three separate aliquots of KRB plus sucrose or dextran blotted on filter paper and a central 5.5 mm button removed with a trephine The corneal button was weighed dissolved in 2 ml Protosol (New England Nuclear Corporation Boston Mass) at 55 C and after cooling and addition of 10 ml of Liquiflour (New England Nuclear Corporation Boston Mass) counted in a Packard Tri-carb liquid scintillation counter One hundred µl aliquots from the endothelial perfusion chamber were similarly treated and counted

The corneal concentration of sucrose or dextran was determined and expressed as µg solute/mg cornea Since it was not possible to measure corneal thickness in this system, corneal endothelial permeability was calculated assuming a corneal thickness of 400 µ and a hydration of 75% The permeability coefficient was calculated using the formula

$$\text{unidirectional flux} = \frac{\text{increase in counts on unlabelled side} \times \text{corneal volume}}{\text{counts on labelled side} \times \text{time} \times \text{area}}$$

Which has the dimensions of cm/sec Results in corneas stored by the two techniques were compared to those values in freshly enucleated rabbit corneas

Results

Sucrose uptake dextran uptake and endothelial permeability in each group of corneas is shown in Table I A comparison of moist chamber eyes and MK corneas after storage for four or ten days shows no statistically significant difference in either corneal levels of labelled sucrose or dextran or endothelial permeability to the different solutes when compared to fresh cornes ($P > 0.08$)

Table I
Cornea endothelial permeability after storage

		Solute uptake (μg/mg cornea)	Permeability coefficient $\times 10^6$ sucrose $\times 10^7$ dextran (cm/sec)*
Sucrose			
Fresh		2.035 ± 0.279	13.69 ± 1.99**
Moist chamber	4 d	1.461 ± 0.135	9.99 ± 0.90
	10 d	1.360 ± 0.160	9.17 ± 1.08
MK medium	4 d	1.779 ± 0.075	12.10 ± 0.51
	10 d	1.760 ± 0.120	11.94 ± 0.79
Dextran			
Fresh		0.235 ± 0.098*	6.40 ± 0.72
Moist chamber	4 d	0.319 ± 0.094	8.39 ± 0.90
	10 d	0.245 ± 0.025	6.13 ± 0.68
MK medium	4 d	0.251 ± 0.465	6.85 ± 1.27
	10 d	0.240 ± 0.095	6.57 ± 0.68

N = 4 all experiments
Assuming corneal thickness 400 μ and hydration 75%
** ± SEM No significant difference within sucrose group or within dextran group $P > 0.05$

Comments

The similar corneal levels of sucrose or dextran at all time periods studied suggests that no alteration of corneal barrier function has occurred in rabbit eyes stored either in moist chambers at 4 C or in rabbit corneas stored in MK medium at 4 C It is known that corneal endothelial permeability to non transported non electrolytes is related to the integrity of the intercellular apical junction rather than to endothelial cell pumping function (Mishima & Trenberth 1968 Kim et al 1971)

It is of interest to note that the endothelial permeability to sucrose and dextran determined in these experiments from corneal uptake of the solutes using assumed corneal hydration and thickness values are close to those values previously reported from determinations made accross the endothelium alone which separated two compartments which were subject to sampling (Mishima & Trenberth 1968 Kim et al 1971) The latter procedure offers greater accuracy due to the sampling from the chambers on each side of the membrane The choice of 400 μ as corneal thickness is certainly valid for fresh tissue as has been noted on numerous

occasions MK stored corneas also show normal thickness (which is the basis for the inclusion of dextran in the medium) The moist chamber corneas however are swollen and the value shown in Table I for moist chamber permeabilities increase by a factor of about 25% with this correction there is no statistically significant difference however between these corrected values and those obtained in fresh corneas Loss of solute across the epithelium is not a factor in these experiments, since this membrane is not permeable to these solutes (Green & Green 1969; Mishima & Hedbys 1967)

Previous work has demonstrated that human corneal endothelial permeability to sucrose and urea increased as a function of both time and age of the donor during storage at 4 C in a moist chamber (Hoefle 1969) It has been demonstrated that the endothelial permeability of older donors increased at a more rapid rate than that of younger donors and that the permeability of a 4 month old donor was unchanged after 80 h of storage (Hoefle 1969) The unchanged permeability in healthy rabbit corneas used in this investigation may be compared to the unchanged permeability in the 4 month old human cornea It is currently not known if human corneas stored in MK medium undergo a change in endothelial permeability as do older eyes stored in moist chambers

This investigation is comparable with pervious work comparing moist chamber storage with storage in MK medium (McCarey ey al 1974) That study demonstrated the ultrastructural integrity of the intercellular space and apical junctions of rabbit endothelial cells after storage in either moist chamber or MK medium. Indeed it appears that both ultrastructurally and physiologically the rabbit endothelial cell intercellular junction deteriorates late in the storage process and is unchanged at ten days in rabbit eyes stored in moist chambers or rabbit corneas stored in MK medium The present data compares with that found for the passive bicarbonate flux (endothelial to stromal surface) across the endothelium of corneas stored as whole eyes in a moist chamber or as isolated corneas in MK medium. In MK solution no change occurred in the measured passive bicarbonate permeability after seven days storage and in moist chamber stored corneas the value increased by about 40% The passive movement of this ion therefore also indicates that little change occurs during storage (Hull et al 1979)

Acknowledgments

Supported in part by Research Grants EY 02386 (DSH) and EY 01413 (KG) from the National Eye Institute in part by a Research Grant from the Georgia Lions Lighthouse Foundation and in part by the Lions Clubs of Augusta Georgia

References

ligar F Kaufman H E McCarey B E & Binder P S (1974) Improved corneal storage for penetrating keratoplasties in man Amer J Ophthal 79 115–120

Dikstein S & Maurice D M (1972) The metabolic basis to the fluid pump in the cornea J Physiol (Lond) 221 29–41

reen K & Green M A (1969) Permeability to water of rabbit corneal membranes Amer J Physiol 217 635–641

Hoefle F B (1969) Human corneal donor material In vitro studies Arch Ophthal (Chicago) 82 361–367

Hull D S Green K & Buyer J (1979) Cornea endothelial bicarbonate fluxes following storage in moist chamber MK medium and MK medium with added hydrocortisone Invest Ophthal 18 484–489

Kim J H Green K Martinez M & Paton D (1971) Solute permeability of corneal endothelium and Descemet s membrane Exp Eye Res 12 231–238

McCarey B E & Kaufmen H E (1974) Improved corneal storage Invest Ophthal 13 165–173

McCarey B E Sakimoto T & Bigar F (1974) Ultrastructure of MK and refrigerated moist chamber stored corneas Invest Ophthal 13 859–863

Mishima S & Hedbys B O (1967) The permeability of the corneal epithelium and endothelium to water Exp Eye Res 6 10–32

Mishima S & Trenberth S M (1968) Permeability of the corneal endothelium to non electrolytes Invest Ophthal 7 34–43

Stark W J Maumenee A E & Kenyon K R (1975) Intermediate term storage for penetrating keratoplasty Amer J Ophthal 79 795–802

van Horn D L Schultz R A & DeBruin J (1975) Endothelial survival in corneal tissue stored in MK medium Amer J Ophthal 80 642–647

Author s address

David S Hull M D Department of Ophthalmology
Medical College of Georgia Augusta Georgia 30912 USA

Department of Ophthalmology (Head S Vannas)
University of Helsinki Helsinki Finland

CORNEAL ENDOTHELIAL CELL DENSITY AFTER AN ATTACK OF ACUTE GLAUCOMA

BY

KIRSI SETÄLÄ

The corneal endothelial cells of 25 patients with unilateral acute glaucoma were photographed with a clinical specular microscope Photography was performed as soon as (average 6–12 h) the IOP had been lowered with a Acetazolamide and/or Mannitol and topical Pilocarpine therapy and the corneas had become clear Peripheral iridectomy was performed on the affected eye and prophylactically on the fellow eye in most of these patients. The follow up endothelial photographs were taken 6–24 months postoperatively

High intraocular pressure lasting 3 days or more lowered the central endothelial cell density But a rise in pressure lasting from only a few hours to 2 days did not affect the endothelial cell count Operative glaucoma procedures caused a loss of central endothelial cells of approximately 4 8% in the series There was a clear correlation between the duration of elevated pressure and the number of central corneal endothelial cells lost

Key words acute glaucoma – corneal endothelial cell density – corneal endothelial cell loss – specular microscope

High intraocular pressure may lead to dysfunction of the corneal endothelium, which permits excess fluid to enter the corneal layers from the anterior chamber It is not clear whether this is due to the high intraocular pressure itself or to secondary damage to endothelial cell function (Stocker 1971)

The endothelial cells of the patient can be examined and photographed with a clinical specular microscope (Maurice 1968 Laing et al 1975 Bourne & Kaufman 1976) In our previous studies with a specular microscope we observed that h... pressure led to a reduction in human central corneal endothelial cell density ...

Received April 9 1979

unilocular capsular glaucoma the raised intraocular pressure affected the central endothelial cell count (Vannas et al 1977). In patients with uniocular Posner-Schlossman syndrome the loss of endothelial cells seemed to be due mainly to the recurrent attacks of raised pressure (Setala & Vannas 1978).

The purpose of this clinical study was to examine the effect of raised IOP attacks on the corneal endothelial cell density in eyes with acute glaucoma. We examined the relation of the endothelial cell density to the duration of the high pressure attacks and the pressure level. The effect of surgical trauma on endothelial cell density was also studied.

Table I

Characteristics of the patients with an acute attack of glaucoma

Case No	Age (years)	Sex	IOP on admission (mmHg)	Duration of attack	Endothelial cell densities		
					affected eye (cells/mm²)	fellow eye (cells/mm²)	difference (%)
1	64	f	64	3 days	[illegible]60	1[illegible]3	63
2	78	f	62	14 days	828	2153	62
3	80	f	66	10 days	766	1863	59
4	57	f	62	7 days	14[illegible]0	2512	41
5	72	f	74	7 days	2484	2898	14
6	80	m	44	2 days	1[illegible]02	218[illegible]	9
7	74	m	58	1 day	2103	2241	6
8	49	m	74	1.5 days	[illegible]015	2[illegible]11	5
9	61	f	60	1 day	2407	2[illegible]18	4
10	50	f	52	2 days	2760	2870	4
11	62	f	63	1 day	18[illegible]2	1904	4
12	66	m	48	2 days	2822	2905	3
13	58	f	68	7 *hours*	2622	26[illegible]0	[illegible]
14	70	f	64	1.5 days	2614	2656	2
15	64	f	51	1 day	2379	2407	1
16	70	m	80	12 hours	2291	2318	1
17	60	f	78	12 hours	2[illegible]05	2[illegible]32	1
18	65	m	76	2 days	2567	2[illegible]67	0
19	65	f	55	1 day	2650	26[illegible]0	0
20	80	f	80	1 day	2152	21[illegible]2	0
21	66	f	70	7 hours	2153	2153	0
22	[illegible]4	f	80	10 hours	2158	2158	0
23	57	f	55	20 hours	2[illegible]88	2[illegible]60	−4
24	71	f	80	1 day	2241	2283	−2
25	65	f	68	1.3 days	2650	2567	−3
Mean	66.3		65.3	2.5 days	2161.4	2392.3	9.7

Material and Methods

The series consisted of 25 patients from Helsinki University Eye Clinic (Table I) 19 females and 6 males All but one of the patients had the first acute attack in the affected eye This patient (No 8) had suffered from an attack of acute glaucoma of two days duration in the same eye 20 years earlier The mean age of the patients was 66 3 years Seventeen patients had the glaucoma attack in the right eye and eight patients in the left eye

The ocular pain blurring of vision vomiting and nausea had lasted three days or longer in five of these patients The history was shorter for the others A careful eye examination was performed on all patients the IOP was measured with a Goldmann applanation tonometer

All the fellow eyes had normal ocular pressure values and no symptoms with no blurring of vision or ocular pain These eyes were therefore used as controls.

Owing to the increased intraocular pressure the corneas were oedematous when the patients were admitted Visualization of the endothelial cells is not possible when the pressure is high and the cornea hazy The endothelial photographs were taken when the intraocular pressure had been lowered with intravenous (Aceta-zolamide or Mannitol) or local (Pilocarpine) therapy

Peripheral iridectomy was performed within 4–6 days of the pressure attack prophylactic surgery was also performed on the normotensive fellow eyes where the angle was narrow Trabeculectomy was performed in the affected eye in only two of the patients If the values for the coefficient of the facility of outflow (C) were under 0 15 a filtering operation was regarded as necessary The endothelial cells were also photographed 6–24 months postoperatively when possible

The series included one patient (No 22) who refused surgery and received no therapy after the acute attack In spite of this the pressure remained normal in both eyes The endothelial photographs were taken two years after the acute attack

Procedure for photography of the endothelial cells with a specular microscope

Several pictures six on average were taken of the central cornea in both eyes of each patient The endothelial cells were analyzed and counted as described in a previous article (Vannas et al 1977)

Results

The average endothelial cell count in the affected eye was 2161 ± 635 and in the fellow eye 2392 ± 346 SD The mean difference was 9 7% ($P > 0\ 05$) (*Table I*)

The intraocular pressure in the affected eye when first measured in the hospital

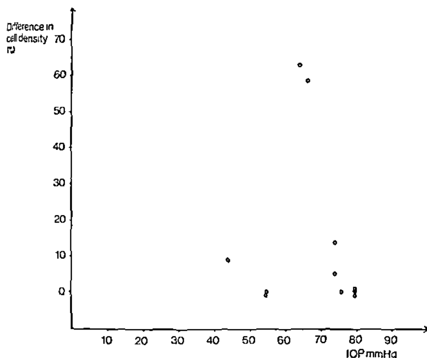


Fig 1

Effect of the level of IOP on the difference in endothelial cell density between the affected and control eyes

(Table I) ranged from 44 mmHg to 80 mmHg mean 65 3 mmHg There was no correlation between the level of IOP and the difference in endothelial cell density (Fig 1)

Effect of duration of glaucoma attack on endothelial cell count

The duration of the attack varied from 7 h to 14 days In five patients the glaucoma attack lasted between 3 days and 2 weeks The endothelial cell density of these five patients was clearly lower in the affected eye than in the normotensive fellow eye (Table I) When the pressure attack lasted less than three days the endothelial cell count was only slightly lower than or about the same as in the control eye Thus there seems to be a clear correlation between the endothelial cell loss and the duration of the glaucoma attack (Fig 2)

The difference in endothelial cell count between the affected and control eye was greatest in a 64 year-old woman (patient No 1 Table I) whose glaucoma attack lasted three days The endothelial cell density in the acutely affected eye was 80 mm² the difference is 63% (Fig 3)

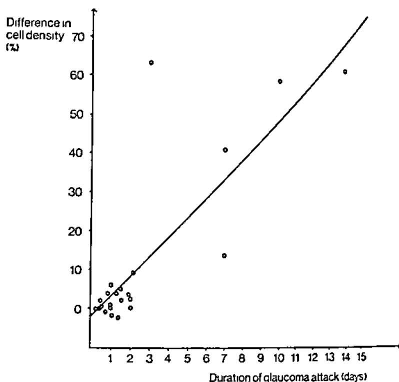


Fig. 2
Effect of the duration of the glaucoma attack on the difference in endothelial cell density between the affected and control eyes

No patient displayed any cupping of the disc or visual field defects during the follow up

In some patients the visual acuity was affected by cataract or macular degeneration Endothelial cell loss did not correlate to visual acuity

Effect of operative procedure on endothelial cell density

The endothelial cell loss after iridectomy was 1 9% in the affected eye and 4.6% in the fellow eye (Table II)

Trabeculectomy was performed on the affected eye in two patients and the mean difference between the pre and postoperative values in their affected eye was 5% In the unoperated fellow eye the cell loss seemed to be 0 5% during the observation period (Table III)

The cell loss caused by glaucoma surgery seems to occur during or soon after the operation No distinct decrease in endothelial cell densities was noted during the 6–24 month postoperative period

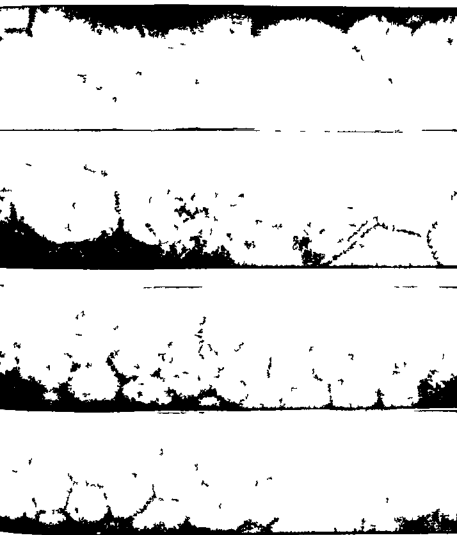


Fig 3

64 year-old woman with 3 days raised IOP (patient No 1) Above the affected eye with 580 cells/mm² below the control eye with 1573 cells mm²

Table II

Effect of operations on endothelial cell density
Endothelial cell density after iridectomy on both eyes

Case No	Affected eye			Fellow eye			Time from operation (m...)
	Before oper	After oper	Diff (%)	Before oper	After oper	Diff (%)	
1	580	538	7	1573	1573	0	19
3	766	621	19	1863	1892	2	8
4	1490	1490	0	2519	2318	8	11
5	2484	2318	7	2898	2650	9	19
8	2015	1964	3	2117	2070		
9	2407	2407	0	2518	2518	0	13
11	1822	1822	0	1904	1904	0	19
12*	2822	2490	12	2905	2905	0	1
13	2622	2277	13	2670	2346	12	6
14	2614	2443	7	2656	2573	3	10
18	2567	2443	5	2567	2360	8	
19	2650	2614	1	2650	2484	6	1
20	2152	2070	4	2152	1987	8	1
21	2153	2153	0	2153	153	0	22
23	2788	2545	9	2760	2 67	7	15
24	2241	2075	7	2283	2213	3	6
25	2650	2573	3	2567	2 67	0	22
mean	2125	2022	4 9	2365 9	2256 6	4 6	13

* iridectomy only on affected eye

Discussion

A rise in pressure lasting at least three days caused a clear decrease in the endothelial cell density of the eye affected by the acute glaucoma attack as compared with the normotensive fellow eye In eyes in which the duration of raised pressure was shorter from a few hours to two days the differences in endothelial cell density between the affected and control eyes were quite small

Because of the small number of patients statistical analysis is not possible The exact duration of raised IOP which causes cell death is perhaps quite variable in different patients It may vary depending on the age of the endothelium which is affected by the pressure and also on the primary cell density before IOP attacks

Glaucoma surgery peripheral iridectomy and trabeculectomy have been suggested to cause lowering of central endothelial cell counts In this series the mean

Table III

Endothelial cell density after trabeculectomy on affected eye

Case No	Affected eye			Fellow eye			Time from operation (months)
	Before oper	After oper	Diff (%)	Before oper	After oper	Diff (%)	
6	1992	1909	4	2185	2111	3	14
15	2379	2241	6	2407	2460	-2	12

No operation

Case No	Affected eye	Fellow eye	After 24 months		Diff (%)	
			Affected eye	Fellow eye		
22	2153	2153	2153	2153	0	o a

loss of endothelial cells after iridectomy was 4 8% and after trabeculectomy about the same Any procedure involving surgical entry into the eye seems to cause damage to the endothelial cells

Today patients with a history of high IOP lasting more than a few days are rare In hospital the pressure can be lowered within hours with drugs (Pilocarpine iv Acetazolamide iv Mannitol)

Endothelial photography was performed soon after admission Two possibilities were envisaged The first was that the cells damaged by the acute glaucoma attack would not yet have diappeared and would still appear morphologically normal in the micrograph The other possibility was that the damaged cells would have died and been resorbed soon after the damage caused by the increased intraocular pressure but that the cornea would not become clear until the remaining living endothelial cells had spread and refilled the gaps left by the dead cells

Follow up photography was done 6–24 months postoperatively The control values were still valid because iridectomy had been performed on both eyes (except in one case) *for bilateral narrow angles This follow up photography showed that* the endothelial cell loss caused by increased intraocular pressure occurs during the glaucoma attack or at least before the cornea becomes clear No distinct cell loss caused by the glaucoma attack was seen during the subsequent follow up period of 6 to 24 months

Recent studies have investigated the extent to which photographs of the central cornea is representative of the entire cornea. In Blackwell's study (1976) specular micrographs were taken in four areas of the cornea and the central cornea provided a measurable indication of the general endothelial cell population in normal young adults, normal older adults and post-cataract adults. Only in the artificial intraocular lens group was there a statistically significant difference in cell numbers in different areas.

In acute glaucoma, however, we may assume that the detrimental effect of the IOP affects the whole cornea uniformly and that the situation is quite different from that obtaining after a cataract operation. Therefore, after an acute glaucoma attack and also after iridectomy, the central endothelial cell density should well represent the endothelial cell number.

There was one patient (patient No. 8) in our series whose case history included an acute attack of glaucoma in the same eye 10 years earlier. However, the difference between the cell densities of the two eyes was small. Casually the endothelial cells seem quite resistant to high and long lasting IOP attacks (Patient No. 5).

The greatest difference in cell density between the affected and control eyes was found in a 64 year old woman after a glaucoma attack lasting three days. It is noteworthy, however, that the endothelial cell count in the control eye was the lowest in the series. It may be assumed that this patient had some additional disorder of the endothelium and that diseased endothelial cells are more vulnerable to the traumatic effect of intraocular pressure.

It seems from this study that high IOP can lower the endothelial cell density in as little as three days. A pressure elevation lasting only from a few hours to two days does not affect the endothelial cell count. For example, the 74 year-old woman with a 10 hour attack (patient No. 22) who refused surgery had no endothelial cell loss after two years follow—up.

These results are in accord with our previous studies on patients with Posner-Schlossman syndrome. Patients with numerous and long lasting attacks of this disease had a lower endothelial cell density in the affected eye than in its fellow (Setala & Vannas 1977).

Glaucoma procedures caused a reduction in central endothelial cell density of approximately 5% in our series.

We conclude that the duration of elevated IOP is a more important factor in damage to the endothelium than the height of the pressure.

References

Ba kwell W L, Cravenstein N & Kaufman H E (1977) Comparison of central corneal endothelial cell numbers with peripheral areas *A ner J Ophthal 84* 4/3–4 b

Bourne W M & Kaufman H E (19/6) Specular microscopy of human corneal endothelium in vivo *Amer J Ophthal 81* 319–323

Lang R A Sandstrom M M & Leibowitz H M (197b) In vivo ph otomicrographs of the corneal endothelium *Arch Opl thal (Chicag) 93* 143–14b

Maurice D M (1)68) Cellular membrane activity in the corneal endothel um of the eye *Experientia (Ba el) 24* 10 4–109b

Setala K & Vannas A (19/8) Endothelial cells in the glaucomato-cyclitic crisi *Adv Ophth l 36* 218–224

Stocker F W (1971) *The en l thelium f the cornea an l its li ical implic t n* 2nd edition pp 13–16 Thomas Springfield Illinois

Vannas A Setala K & Ruusuvaara P (1977) Endothelial cells in capsular glaucoma *Acta Ophthal (Kbh.) 55* 9b1–9b8

Author s add

Kirsi Setala M D The University Eye Clinic

University of Helsinki SF 00290 Helsinki 29 Finland

Department of Ophthalmology (Head: N. Ehlers)
Århus Kommunehospital, University of Aarhus, Denmark

VARIATIONS IN ENDOTHELIAL MORPHOLOGY OF NORMAL CORNEAS AND AFTER CATARACT EXTRACTION

A specular microscopic study

BY

THOMAS OLSEN

The central corneal endothelium of 22 normal subjects and 11 unilateral aphakic subjects was photographed with the non-contact specular microscope. The aphakic patients had undergone intracapsular cataract extraction one to four years previously. Endothelial cell densities were estimated. All cells which had been counted were then grouped according to their number of neighbours. The percentages of cells having 4, 5, 6, 7 or 8 neighbours were (means ± SD): 0.4 ± 0.8, 18.0 ± 3.4, 64.1 ± 6.5, 15.9 ± 2.6 and 0.9 ± 0.8 per cent, respectively in the normal eyes, and 0.8 ± 1.3, 20.9 ± 3.7, 57.4 ± 5.6, 19.6 ± 3.9 and 1.4 ± 1.2 per cent in the aphakic group, respectively. The frequency of cells having 5, 6, or 7 neighbours were significantly different in the two groups. Cells with 9 or 10 neighbours were not seen in the normal group but occurred in 2 and 1 of the aphakic eyes, respectively. In the normal group the frequency of hexagonal cells was found to correlate to the cell density ($r = 0.46$, $2P < 0.01$) and age ($r = -0.40$, $2P < 0.05$).

Key words: cornea – endothelium – morphology – specular microscopy

The endothelium of the normal cornea comprises a coherent monolayer of large hexagonal cells, the integrety of which is essential for a normal hydration of the cornea. Following various forms of mechanical injury, the endothelium has been stated to recover and restore a normal cellular pattern from one week to three months in the rabbit (Chi et al. 1960; Khodadoust & Green 1976; Olson et al. 197-; van Horn et al. 1977). Longer time periods have been observed for the ca

Received May 1, 1979.

endothelium presumably more comparable to man in its limited proliferative power (van Horn et al 1977)

If human endothelial cells are damaged immediate healing occurs by spreading and sliding of undamaged cells to cover the defect Since the proliferative power of the human endothelium is very limited (Kaufman et al 1966) the result is a decrease in the cell density Among the factors which cause a decrease in cell density is age (Laing et al 1976 Bourne & Kaufman 1976 Sturrock & Sherrard 1978 Olsen 1979) and cataract extraction (Bourne & Kaufman 1976 Hirst et al 1977 Cheng et al 1977 Forstot et al 1977)

This study was undertaken in order to investigate whether cell loss leaves permanent changes in the cellular mosaic of the human endothelium A knowledge of such morphological alterations may be of help in other clinical situations when cell loss is suspected but the cell density is not obviously lowered

Subjects and Methods

The central corneal endothelium of one eye of 22 normal subjects and both eyes of 11 unilateral aphakic patients were photographed with the non-contact specular microscope (Olsen 1979) The normal subjects comprised medical students members of the medical staff or patients with senile lens opacities The age range was 19–83 years The aphakic patients had intracapsular cataract extraction one to four years previously They were photographed successively as they came to have surgery performed on the other side Age range 38–89 years None of the normal or the aphakic patients showed any signs of present disease in the cornea.

Endothelial cell densities were estimated as described earlier (Olsen 1979) All cells which had been counted were then grouped according to their number of neighbours The relative distribution of cells according to their number of neighbours was calculated for each cornea

Results

For the total group of normal eyes estimated endothelial cell densities ranged from 1 10 to 3575 cells/mm² A negative correlation was found to the age of the subject ($r = -0 31$ $0 1 > 2P > 0 05$ $n = 33$) Mean estimated cell densities from the aphakic patients appear from Table I The difference in cell counts between unoperated and operated eyes ranged from −8 7 to −67 1%

The percentages of cells having 4 5 6 7 or 8 neighbours were (means ± SD) 1 4±0 8 18 0±3 4 64 1±6 5 15 9±2 6 and 0 9±0 8 per cent respectively in the normal eyes and 0 8±1 3 20 9±3 7 57 4±5 6 19 6±3 9 and 1 4±1 2 per cent in the aphakic group respectively (Fig 1) The aphakic eyes had significantly

Table I
Endothelial cell density of 11 unilateral aphakic patients
(means ± SD)

Normal eye (cells/mm²)	Aphakic eye (cells/mm²)	Difference ( )
2736 ± 399	1749 ± 697	−36 8 ± 16 3

higher frequency of cells with 5 and 7 neighbours and lower frequency of cells w J 6 neighbours Cells with 9 or 10 neighbours were not seen in the normal group bu occurred in 2 and 1 of of the aphakic eyes respectively

A significant correlation was found between the number of hexagonal cells an the cell density in the group of normal eyes (Fig 2) (r = −0 40 0 01 > 2*P* > 0 001) weaker correlation was found between the number of hexagonal cells and ag (r = −0 40 0 05 > 2*P* > 0 01) (Fig 3)

In the aphakic group the number of hexagonal cells could not be correlated t either time after the operation endothelial cell density or difference betwe counts from operated and unoperated eye

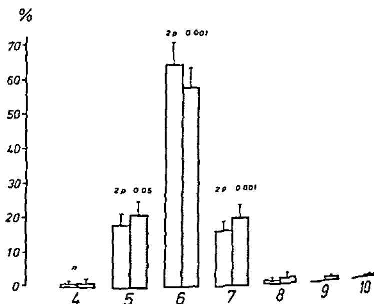
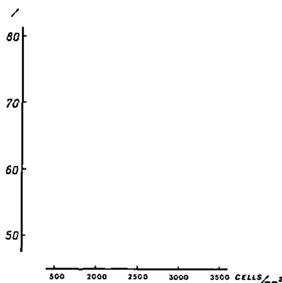


Fig 1
Relative frequency of endothelial cells having 4 to 10 neighbours in 53 normal eyes (h columns) and 11 cataract extracted eyes (cro hatched columns) Vertical bar Statistics unpaired *t* test



F g 2
C rrelation between the relative number of hexagon l cells and the cell densit in 33 normal eyes (r = 0 46 0 01 > 2P > 0 001)

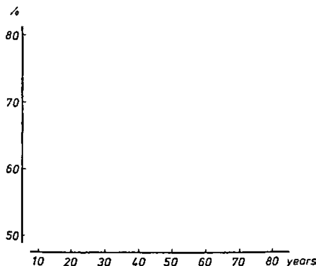


Fig 3
Correlation betv een the relative number of hexagonal cells and age (r = −0 40 0 05 > 2P > 0 01 n = 33)

Discussion

The present study has shown that a normal cellular pattern is not reformed [illegible] to four years after injury to the human endothelium. Normally a high concordance exists between endothelial cell counts from left and right eyes (Olsen 1979). The cell loss which occurred at time of cataract extraction is therefore indicated in the difference in counts between the operayed and unoperated fellow eyes (Table I).

The finding of a decrease in the number of hexagonal cells with decreasing cell density indicates that the gradual cell loss during aging also leaves permanent changes in the original largely hexagonal cell pattern. Since age was found to be a minor determinant for the variation in the number of hexagonal cells, this result adds further evidence to a hypothesis that cell loss varies from one individual to another during aging. In other words, the large interindividual variation in cell density seen in old age (Olsen 1979) is an acquired variation and not only a variation present at birth.

The reason why the hexagon is the dominant shape of endothelial cells is unknown. From a geometrically point of view the hexagon is one of the three regular tessalations which can be repeated to cover the whole plane (see Coxeter 1973), but a triangle and a square could do this as well. It can only be speculated that the characteristics of the hexagon, i.e. its almost circular shape which keeps the total perimeter at a minimum and with neighbouring faces meeting only three at a time, is favorable to cells which must function as a barrier against the stromal imbibition of water.

Acknowledgments

The technical assistance of Mrs. Anette Poulsen is gratefully acknowledged. This work was supported by grants from Fonden til Lægevidenskabens Fremme.

References

Bourne W. M. & Kaufman H. E. (1976) Specular microscopy of human corneal endothelium in vivo. *Amer J Ophthal 81* 319–323.

Bourne W. M. & Kaufman H. E. (1976) Cataract extraction and the corneal endothelium. *Amer J Ophthal 82* 44–46.

Cheng H., Sturrock G. D., Rubinstein B. & Bulpitt C. J. (1977) Endothelial cell loss and corneal thickness after intracapsular extraction and iris clip lens implantation: a randomized controlled trial. *Brit J Ophthal 61* 78?–790.

Chi H. H., Teng C. C. & Katzin H. M. (1960) Healing process in the mechanical denudation of the corneal endothelium. *Amer J Ophthal 49* [illegible].

Coxeter H. S. M. (1973) *Regular polytopes* p. 58. Dover Publications Inc., New York.

Forstot S L. Blackwell W L. Jaffe N S & Kaufman H E (1977) Effect of intraocular le implantation on corneal endothelium *Trans amer Acad Ophthal Otolaryngol 83* 195–203

Hirst L W Snip R. C Stark W J & Maumenee A E (1977) Quantitative corne endothelial evaluation in intraocular lens implantation and cataract surgery *Amer Ophthal 84* 775–780

Kaufman H E. Capella J A & Robbins J E (1966) The human corneal endothelium *Am J Ophthal 61* 835–841

Khodadoust A A & Green K (1976) Physiological function of regenerating endotheliu *Invest. Ophthal 15* 96–101

Laing R. A Sandstrom M M Berrospi A R. & Leibowitz H M (1976) Changes in th corneal endothelium as a function of age *E p Eye Res 22* 587–594

Olsen T (1979) Non-contact specular microscopy of human corneal endothelium *Ac ophthal (Kbh.) 57* 986–998

Olson L E Marshall I Rice N S C & Andrews R (19 8) Effects of ultrasound on th corneal endothelium II The endothelial repair process *Brit J Ophthal 62* 145–154

Sturrock G D Sherrard E. S & Rice N S C (1978) Specular m croscopy of the corne endothelium *Brit J Ophthal 62* 809–814

Van Horn D L Sendele D D Seideman S & Buco P J (1977) Regenerative capacity of th corneal endothelium in rabbit and cat. *Invest Ophthal 16* 597–613

Author s add ess

Thomas Olsen Department of Ophthalmology
Århus Kommunehospital DK 8000 Århus C Denmark

Department of Ophthalmology (Head Salme Vannas)
University of Helsinki Helsinki

CORNEAL ENDOTHELIAL CELLS IN ESSENTIAL IRIS ATROPHY

A specular microscopic study

BY

KIRSI SETALÄ and ANTTI VANNAS

The specular microscope was used to study the corneal endothelium in three patients with essential iris atrophy and three patients with anterior mesodermal disorders with uncertain diagnose Pleomorphic and enlarged endothelial cells were found to be typical for essential iris atrophy The changes were also present in one eye with normal pressure
In the patients with other types of iridal disorder the endothelial cells were normal in form In one of these patients high intraocular pressure and surgery had seemingly led to enlargement of the endothelial cells
According to our findings the specular microscope is a useful aid in differentiating essential iris atrophy from other disorders of the iris

Key words essential iris atrophy – corneal endothelial cells – specular microscopy – Chandler s syndrome – iridoschisis

Essential atrophy of the iris (EIA) is a rare disease of unknown aetiology characterized by slowly progressive atrophic changes in the iris trabecular meshwork and corneal endothelium and is often accompanied by glaucoma due to changes in the chamber angle (Duke Elder 1966)

Zentmeyer (1918) suggested that vascular sclerosis may be the cause of essential iris atrophy Heath (1953) expanded this theory with histopathological studies and demonstrated that in far advanced cases the mechanism for the atrophy lies in multiple anaemic infarctions that follow segmental vascular occlusions However L

Received August 28 1979

Fig 1 (case 1)
Left eye of the 35 year-old woman with essential iris atrophy of 6 years duration

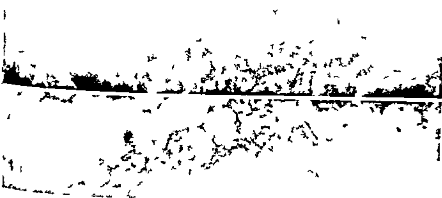


Fig 1A
Top Hazy abnormal endothelial cells of the affected left eye of the 35 year-old woman with essential iris atrophy (× 440)
Bottom Contralateral healthy eye with endothelial cells of normal appearance (2800 cells/mm²) (Contact specular micrograph × 440)



Fig 2 (case 2)
Right eye of the 42 year-old woman with essential iris atrophy Duration 25 years

Vannas (1969) using iris angiography to examine eyes with essential iris atrophy found no specific pathological vascular changes

Recently the hypothesis has been proposed that in essential iris atrophy important roles are played by corneal endothelial degeneration and the growth and contraction of an ectopic endothelial membrane (Yanoff & Fine 1975 Scheie et al 1976 Rodrigues et al 1978) Campbell et al (1978) have proposed a new designation for the syndrome primary proliferative endothelial degeneration

In view of the lack of information on the state of the corneal endothelial cells in vivo in this syndrome we present data on patients with typical essential iris atrophy Also three patients with suspicious EIA are presented The specular photography excluded the diagnosis

Patients and Methods

Our series is presented in Table I In addition to the regular ophthalmological examination the corneal endothelium was photographed with a contact specular microscope (Bourne & Kaufman 1976) and a non-contact specular microscope bas

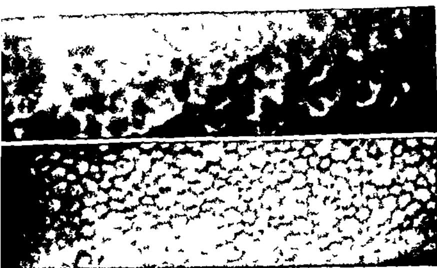


Fig 2A (case 2)

Top The endothelial cells of the diseased right eye The outlines of the cells are poorly seen × 130)

Bottom Endothelial cells of the contralateral healthy eye (3000 cells mm²) (non contact specular micrograph × 130)

by one of us (A V) With the non-contact method the primary magnification on the film is ×34 allowing inspection of a larger endothelial area up to 500 cells in a single picture

Results

A 3?-year-old woman (Fig 1 Table I case 1) noticed an extra pupil in her left eye at the age of ?9 and the diagnosis of essential iris atrophy was made a year later The family and medical histories were negative there being only a history of refractive error General physical examination revealed no abnormalities Follow up examination in 19?8 revealed normal vision in the right eye The right eye was normal With the best correction the vision in the left eye was 0 5 Biomicroscopy showed an endothelium with a fine beaten silver appearance The anterior chamber was normal in depth The pupil was markedly distorted The iris was thin with stretched holes one strand lying across the middle of the cornea Gonioscopy revealed broad anterior synechiae During follow up the eye has remained normotensive Endothelial photographs were taken of both eyes Fig 1A shows the endothelial cells of the affected left eye with hazy irregular pleomorphic cells The contralateral healthy eye of the same patient showed a normal regular mosaic like cell pattern

The second patient a 49 year-old woman noticed at the age of 17 that the pupil of her right eye was distorted towards the 2 o clock direction (Fig 2) Essential iris atrophy of the right eye was diagnosed in 1959 At follow up examination in 19 6 the ocular tension was normal but the iris atrophy showed slow progression In April 19 9 examination howe?

normal corrected visual acuities in both eyes The right pupil was distorted and there was a hole in the iris at the 10–11 o clock position Gonioscopy revealed relative angle closure and large synechiae An elevated IOP of 40 mmHg was measured but was normalized with medication The optic nerve head was normal The left eye was normal In micrographs the corneal endothelial cells in the right eye were poorly seen The cells seemed to be large and their images hazy The endothelial cells of the left eye were normal (Fig 9A)

The third patient a 63 year-old woman noticed at the age of 56 that the pupil of her left eye was displaced (Table I case 3) The diagnosis of essential iris atrophy was made at the age of 57 (1972) when there were two holes in the left iris and elevated IOP (41 mmHg) in the left eye The C value was 0 07 (μl/min/mmHg) and therapy for glaucoma was started (pilocarpine and adrenaline) Follow up examination in 1978 revealed normal vision in the right eye The IOP was 16 mmHg and biomicroscopy revealed no abnormality The vision of the left eye was 0 25 owing to macular degeneration There were three holes in the iris Gonioscopy showed broad adhesions in the areas corresponding to the holes and elsewhere the angle was open The IOP of the left eye was 30 mmHg Endothelial photographs were taken of both eyes The endothelial cells in the affected eye appeared somewhat hazy and the cell density could not be counted In the healthy fellow eye the endothelial cells were regular their density being 2900 cells/mm^2

The fourth case is a 60-year-old woman (Fig 3) From the age of 41 she sometimes noticed dimness in her left eye in the evenings At that time her left iris became lighter in colour Three years later secondary glaucoma was diagnosed in the left eye Visual acuity in both eyes was 1 0 with glasses Biomicroscopy showed some coalescent guttata in the left cornea The

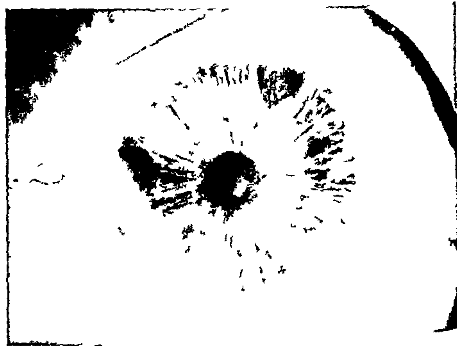
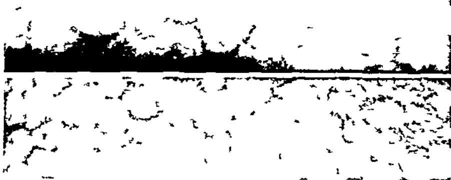


Fig 3
Left eye of the 60 year-old woman with iridoschisis of 20 years duration



F g 3 A

Top Large endothelial cells in eye with iridoschisis (650 cells mm²) The lowered cell density as probably caused by high IOP and intraocular surgery (× 440)

Bottom The endothelial cells of the healthy contralateral eye of the same patient (3000 cells mm²) (× 440)

anterior chamber was shallow The colour of the left iris was lighter and there was stromal atrophy Gonioscopy showed angle closure with broad irregular iridal adhesions The right anterior segment was normal The visual fields were free from any scotomas Ophthalmoscopy showed neither abnormalities nor disc asymmetries The intraocular pressures were 45 mmHg in the left eye and 16 mmHg in the right eye Medication with pilocarpine and adrenaline was initiated In November 19"4 (at the age of 56) trabeculectomy was performed on the left eye Since then the intraocular pressure has been normal without medication. The ocular findings in this patient simulate those of iridoschisis Iridoschisis usually begins in the th decade It is usually bilateral but may be unilateral. There is localized clea age of the mesodermal iris stroma into two layers the anterior leaf separating and disintegrating into fibrils, the distal ends of which float freely in the anterior chamber (Duke—Elder 1966) Endothelial photographs of both eyes were taken in January 19"9 and in the right eye the endothelial cells were normal (3000 mm²) In the left eye the endothelial cells were ery large but quite regular and sharply visualized (650 cells mm²) (Fig 3 A)

At the age of 19 the fifth patient noticed that her left pupil had become oval (Fig 4) In photographs taken at the age of 15 both pupils were round and symmetrical. The family history was non-contributory and she denied any ocular trauma. At the age of 22 she was examined at Helsinki University Eye Clinic Visual acuity in both eyes was 1 0 and gonioscopy revealed that the angle was closed at the 8–9 o clock position, with broad adhesions in the left eye In April 19"9 at the age of 34 the isual acuity in both eyes was 1 0 the ocular pressures were 15 mmHg o a. and the left pupil was o al as in the photos taken 12 years earlier The gonioscopy finding was also unchanged The pupil of the right eye was normal. The micrographs show that the endothelial cells in the two eyes are similar (Fig 4 A)

Our last patient is a 38-year-old woman whose left pupil had been oval for as long as she can remember For the last ten years she had been followed up at our clinic the last examination being in August 19 8 The visual acuity in both eyes was 1 0 and the right pupil

Fig 4 (case 5)
Left eye of the 34 year-old woman with oval pupil since the age of 19

and iris were normal The left pupil was oval and in the inferior temporal quadrant the pigmented epithelium of the iris was totally absent and some of the stroma was [illegible]. Gonioscopy revealed that the angle was open elsewhere but deformed by anterior [illegible] at the 3–6 o clock position Micrographs of the corneal endothelium showed that the [illegible] of the two eyes were similar and the endothelial mosaic was regular

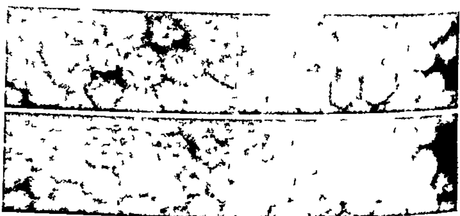


Fig 4A
Regular hexagonal endothelial cells of case No 5 [illegible] left eye ([illegible] cells mm) [illegible] right eye (3000 cells mm) (× 440)

Table I
Signs and symptoms in the patients

Case No	Diagnosis	Age (years)	Duration of disease (years)	Visual acuity in affected/fellow eye	Highest IOP in affected/fellow eye (mmHg)	Endothelial cells	Endothelial cell density (cells/mm²)	
							Affected eye	Fellow eye
1	Essential iris atrophy	[illegible]	6	[illegible]	18/17	Abnormal	–	2800
2	Essential iris atrophy	[illegible]	[illegible]	1.0/1.0	40/16	Abnormal	–	3000
3	Essential iris atrophy	63	7	[illegible]	44/14	Abnormal	–	2900
4	Iridoschisis	60	[illegible]	1.0/1.0	[illegible]	Large	[illegible]	3000
5	Pupillary malform (acquired)	31	1	1.0/1.0	[illegible]	Normal	[illegible]	3000
6	Pupillary malform (congenital)	38	Congenital	1.0/1.0	14/13	Normal	3000	3000

Discussion

The first three cases presented fulfil the criteria of typical essential iris atrophy in all respects. The disease began in an initially normal eye with mild distortion and displacement of the pupil. Next, the pigment layer of the iris began to show defects. Finally, large parts of the iris degenerated and features of secondary glaucoma destruction developed (Alkemade 1969). The description of Rieger's syndrome in the literature dose not quite fit the findings made in any of these six patients. In Rieger's syndrome (dysgenesis mesodermalis of the iris and cornea) the primary dysgenesis is congenital rather than acquired; it is usually stationary rather than progressive and is always bilateral.

In eyes with essential iris atrophy the morphology and size of the corneal endothelial cells can be studied *in vivo* with the specular microscope. In the patients with essential iris atrophy (cases 1, 2, 3) specular micrographs show endothelial cells that are clearly abnormal morphologically and different from those of the normal fellow eyes. Hetherington (1978) found similar abnormalities in endothelial cells in patients with Chandler's syndrome, a probable variant of EIA. With [illegible] electron microscopy Campbell et al. (1978) demonstrated aberrant endothelial cells over the angle and on the iris. The ectopic endothelial membrane has been detected in eyes that have been enucleated because of far advanced changes due to secondary glaucoma or in trabeculectomy and iridectomy specimens. In these previous studies the angle and trabeculum have been examined but not the endothelial cells of the central cornea.

In micrographs of normal corneal endothelial cells the cell surfaces reflect light and so appear light, while the cell boundaries absorb light and appear dark. In essential iris atrophy the endothelial cells of the affected eyes reflect light abnormally, the cell boundaries being lighter and the cells darker. It is known that diseased endothelial cells form additional amounts of Descemet's membrane. Later the thickness of this membrane becomes uneven and the "cobblestone" pattern is formed, which seems to account for the changes seen in the micrographs. Conceivably, these corneal endothelial cells seem to oversecrete collagenous material in the same way as the cells in the chamber angle (Campbell et al. 19[illegible]).

The history and previous examinations of the three patients with essential iris atrophy revealed that the IOP was high in cases 2 and 3, but normal in case 1. The endothelial cell pattern was modified in a similar way in all three patients with EIA. In patients with glaucoma the IOP, although it affects the endothelial cell density, does not modify the appearance of the cells to the extent seen in these patients (Vannas et al. 1977, Setälä & Vannas 1978).

The fourth patient had iridoschisis and the cells, although very large, were

regular and sharply visualized The low cell density may have been induced by th high IOP and the surgical trauma together

The two last patients had stationary iridal disorders and do not fit in essential ir atrophy The endothelial cells were normal in appearance in both eyes and the ce density did not differ between the affected and unaffected eye

Essential iris atrophy is a rare monocular disorder which also involves the cornea endothelium The pathological appearance of the endothelial cells calls for furhe studies with the specular microscope which can be used in differentiating essenti iris atrophy from other iridal disorders

References

Alkemade P P H (1969) *Dysgenesis mesodermalis of the iris and the cornea.* A study of Rieger syndrome and Peter s anomaly Koninkl jke Van Gorcum & Co Assen The Netherlands

Bourne W M & Kaufman H E (1976) Specular microscopy of human corneal endotheliun in vivo *Amer J Ophthal 81* 319–323

Campbell D Shields B & Smith T (1978) The corneal endothelium and the spectrum o essential iris atrophy *Amer J Ophthal. 86* 303–3?4

Duke Elder S & Perkins E S (1966) Diseases of the uveal tract, pp 686–694 Vol IX. In Duke Elder S ed *System of Ophthalmology* Mosby St. Louis

Heath P (1953) Essential atrophy of the iris A histopathologic study *Trans. amer ophthal. So 51* 167–192

Hetherington J Jr (1978) The spectrum of Chandler s syndrome Symposium Glaucoma *Ophthalmologica 85* 240–251

Rodrigues M Streeten B W & Spaeth C. L. (1978) Chandler s syndrome as a variant o essential iris atrophy A clinicopathologic study *Arch. Ophthal (Chicago) 96* 643–659

Scheie H C Yanoff M & Kellog W T (1976) Essential iris atrophy Report of a case *Ar Ophthal (Chicago) 94* 1315–1320

Setala K & Vannas A (1978) Endothelial cells in the glaucomatocyclitic crisis *Ad Ophtha 36* 918–924

Vannas A (1969) Fluorescein angiography of the vessels of the iris in pseudoexfoliation o the lens capsule capsular glaucoma and some other forms of glaucoma. *Acta ophthal. (Kbh Suppl 105*

Vannas A Setala K & Ruusuvaara P (1977) Endothelial cells in capsular glaucoma *Ac ophthal (Kbh) 55* 951–958

Yanoff M & Fine B S (1975) *Ocular Pathology* A text and Atlas Harper & Row Hagerstow Md

Zentmeyer W (1918) Essential atrophy of the iris *Amer J Ophthal. 1* 510

Author s address

Kirsi Setala M D Department of Ophthalmology
University of Helsinki Haartmanikatu 4 C 00290 Helsinki 29 Finland

Department of Ophthalmology (Head: Sven Erik G. [illegible]), Linköping University, Sweden and the Smith-Kettlewell Institute of Visual Sciences, (Head: Arthur Jampolsky), San Francisco, USA

CONTRACTILE PROPERTIES OF EXTRAOCULAR MUSCLE IN SIAMESE CAT

BY

Gunnar Lennerstrand

Siamese cats are albinos with poor visual resolution and severely impaired binocular vision. Eye muscle physiology was studied in Siamese cats as a part of a more extensive project on eye muscle properties in cats with deficient binocular vision. Isometric contractions of the inferior oblique muscle were recorded in response to single and repetitive muscle nerve stimulation. Speed of contraction, measured as twitch contraction time, fusion frequency and rate of tetanic tension rise, was lower in Siamese than in normal cats. Eye muscles of Siamese cats fatigued more easily to continuous activation than normal cat eye muscle. These functional changes have also been found in cats with binocular defects from monocular lid suture, but were much more marked in Siamese cats. It is suggested that the eye muscle changes represent muscular adaptations to genetically caused impairments of binocular vision and visual resolution in Siamese cats.

Key words: extraocular muscle – contractile properties – Siamese cats – binocular deficiency

Albinism is a genetic defect of melanin synthesis, associated with impairment [illegible] visual function, such as low visual acuity, defective binocular vision and [illegible] (Duke-Elder 1963). The neuroanatomical and neurophysiological basis for [illegible] visual abnormalities have been particularly well studied in the Siamese cats ([illegible] et al. 1974). Functional properties of retinal ganglion cells are different in [illegible] and Siamese cats (Chino et al. 1978), but the most profound abnormalities [illegible] Siamese visual function can be related to a misrouting of a part of the [illegible]

Received January 30, 1979

geniculate fibers (Guillery 1969 Guillery & Kaas 1971 Kalil et al 1971 Shatz 1977) These fibers project to the lateral geniculate body on the wrong side and this induces functional adaptations in the visual cortex (Hubel & Wiesel 1971 Cool & Crawford 1972 Kaas & Guillery 1973) as well as in structures like the pretectum and the superior colliculus that act as linkage between visual function and oculomotion (Kalil et al 1971 Berman & Cynader 1972 Lane et al 1974) Recently Collewijn et al (1978) discovered a marked instability in the optokinetic responses of albino rabbits which they suggested was related to abnormal visuomotor connections The purpose of the present study was to investigate if these functional changes in Siamese cats might affect eye muscle properties

For normal binocular functions including stereopsis binocularly responsive visual cortex neurons are a prerequisite (Bishop 1973) In the Siamese cat all neurons in the visual cortex are monocularly driven (Hubel & Wiesel 1971 Cool & Crawford 1972 Kaas & Guillery 1973) and these cats have no stereopsis (Packwood & Gordon 1975) In pigmented cats with normal visual pathways similar changes in striate neuron function can be induced by permanent misalignment of the eyes if the cats have been squinting since they were four weeks of age or younger (Hubel & Wiesel 1965 Yinon 1976) Although convergent squint is of common occurrence in Siamese cats both the strabismic animals and the animals with apparently straight eyes show exactly the same abnormalities of cortical functions (Cool & Crawford 1979 Shatz 1977)

Disruption of binocular vision in normal cats by monocular visual deprivation at an early age was accompanied by changes in eye muscle function It was noted that the speed of contraction and the fatigue resistance were lower in the inferior oblique muscles of these animals than in the muscles of cats with normal binocular development (Lennerstrand & Hanson 1979) It will be shown in the present study that Siamese cats with a genetic defect of binocular vision exhibit the same kind of eye muscle abnormalities but to an even greater extent than the cats with aquired binocular dysfunction

Methods

The experiment were performed on four Siamese cats one of which had esotropia (convergent strabismus) but the rest were apparently non-squinting In all the cats the retina and the choroid completely lacked pigment

The animals were anaesthetized with pentobarbital i p (40 mg kg b w) Previous papers by Hanson & Lennerstrand (1977) and Lennerstrand & Hanson (1978a 1979) have given detailed descriptions on (1) the preparation of the inferior oblique muscle for isometric tension recording to single and repetitive stimulation of the muscle nerve (2) the force sensing system and (3) the stimulus procedures to study speed of contraction in twitch and tetanus fatigue properties and post tetanic potentiation The rapid tension changes were

recorded on a storage oscilloscope (Tektronix 5103 N) and the responses with a slower time course on an ink writer (Beskman Model RB) which was sufficient for resolving the tension changes during fatigue stimulations

The results from six inferior oblique muscles in these four Siamese cats were compared with data from the same muscle in fully pigmented cats with no obvious impediments to normal binocular vision already reported by Lennerstrand & Hanson (1979) t-test for means were used in the statistical analysis

Results

Speed of contraction was significantly lower in the inferior oblique muscles of the Siamese cats than in control animals This was obvious from recordings of twitch as well as tetanic contractions As shown in Table I twitch contraction time (*ct*) was 5. msec on an average in normal cats and 7 4 msec in the Siamese The differences in the half relaxation (*hrt*) of the twitches were even larger 7 1 msec in normals and 11 6 msec in Siamese cats A typical twitch response of a Siamese inferior oblique is shown in Fig 1 The contraction peak and the half relaxation point were reached considerably later than in normal cats (marked by arrows) As for the speed of tetanic contraction significant differences was found between the groups in the rate of tension rise and the stimulus frequency to produce fused tetani (see Table I).

The maximal twitch tension was higher in Siamese than in normal cats (5 4 and 3 7 g respectively) but the maximal tetanic tension was not significantly different in the two groups

On increasing the stimulus strength from threshold to supramaximal values the *ct* and *hrt* decreased steadily i e the slowest contractions were recorded to threshold stimulation and the fastest to supramaximal stimulation This is the same behaviour



Fig 1

Twitch contraction (top trace) to supramaximal nerve stimulation in a Siamese inferior oblique muscle The lower trace carries the stimulus pulse The arrows indicate position of peak twitch contraction (●) and half relaxation (□) of normal cat inferior oblique (see Lennerstrand & Hanson 1979) Twitch contraction time and half relaxation time are longer in Siamese than in ordinary cat eye muscles

as seen in eye muscles of normal cats and probably means that threshold stimulation activated slow units in both Siamese and normal cats (see Lennerstrand & Hanson 1978a)

Fatigue properties were also markedly different in the two groups of cats To continuous stimulation at 100 and 200 Hz the tension drop was much larger in the Siamese then in control cats as seen in Fig 2 The tension remaining after 30 sec of stimulation in percent of the tension at stimulus initiation has been used as a measure on muscle fatigue resistance (endurance) As shown in Table I the average

Table I

Contractile characteristics in which significant differences existed between normal (N) and Siamese (Si) cats Number of muscles (n) in each group as indicated Mean values ± SD are given. Twitch response parameters represented are amplitude (force difference between pre-twitch level and twitch peak) contraction time (*ct*) and half relaxation time (*hrt*) as indicated in Fig 1 Tetanus response parameters presented are fusion frequency i e the lowest stimulus frequency to reach a smooth or fused tension curve and rate of tension rise in the steepest part of the tetanus curve measured as percent of maximal tension (P_O) per millisecond Endurance has been measured as the tension output after 30 sec continous stimulation at 100 or 200 Hz in percent of the tension at stimulus initiation (see also Fig 2) Post tetanic twitch *hrt* in percent of pre tetanic values were determined for twitch responses obtained 5 min after a 30 sec tetanization at the frequencies of stimulation indicated

	N (n = 8)	Si (n = 6)	P
Twitch			
ampl (g)	3 7±1 0	5 4±0 7	
ct (ms)	5 7±0 6	7 4±1 4	
hrt (ms)	7 1±1 0	11 6±3 2	
Tetanus			
fusion frequency (Hz)	331±24	292±25	
rate of tension rise			
(% P_O msec)	5 8±1 6	4 4±1 4	
Endurance (%)			
at 100 Hz	95±15	63±13	
at 200 Hz	47±4	32±8	*
Post tetanic twitch hrt (%)			
at 50 Hz	103±16	145±32	
at 100 Hz	102±10	172±57	**
at 200 Hz	105±22	186±66	

0 05<*P*<0 02 0 02<*P*<0 01 * *P*<0 01

fatigue resistance was 94% at 100 Hz in normal cats while in the Siamese cats endurance as only 63%. At 200 Hz stimulation the values were 4?% for the control and 32% for the Siamese cats. During 50 Hz stimulations the tension of both Siamese and normal inferior oblique showed a steady increase thus displaying a so-called stair case phenomenon. In both groups final tension reached 1 0–140% of the initial value.

As a result of the long stimulations twitch responses showed post-tetanic

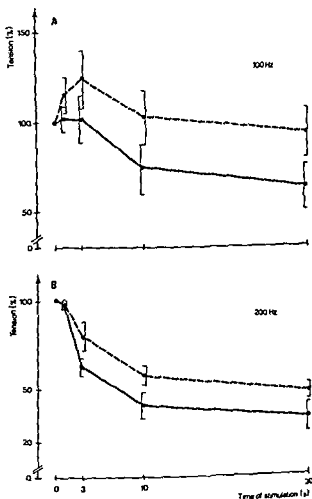


Fig. 2

Graphical display of tension development during continuous stimulation for 30 sec at 100 Hz in A and 200 Hz in B. Values from Siamese cats are connected with full lines and those from normal cats with interrupted lines. Tension has been measured at 1, 3, 10 and 30 sec of stimulation and expressed in percent of the tension at stimulus initiation. Average values ... have been plotted. Fatigue was more pronounced in Siamese than in ordinary cat eye muscle.

depression that lasted up to ten min and the *ct* and *hrt* were prolonged (Lennerstrand & Hanson 1978b) These effects were quantitatively the same in normal and Siamese cats except for *hrt* which was significantly longer in Siamese twitches recorded 2 and 5 min after the fatiguing stimulus

Responses to short tetanic stimulations (200 Hz for 0 5 sec) have been shown to be reduced for approximately two minutes as a result of continuous stimulation for 30 sec (Lennerstrand & Hanson 1978b) Similar effects were obtained in the Siamese group with no significant difference from the normal animals Thus recovery of a tetanus after long muscle activation was equally rapid in both groups

Post tetanic potentiation (PTP) occurred after short tetani (200 or 400 Hz for 1 sec) as reported by Lennerstrand & Hanson (1978b) for normal eye muscles The effects were the same in eye muscles of Siamese cats

Succinylcholine is known to induce contracture in extraocular muscle but flaccid paralysis in normal skeletal muscle (Bach y Rita 1971) Injections of 1 mg/kg b w produced a contracture of 10–15 min duration in both Siamese and normal cats The peak tension obtained with the drug was 30–35% of maximal tetanic tension to nerve stimulation (at fusion frequency) in both groups of animals

Discussion

Eye muscles of Siamese cats showed slower contractions and fatigued more easily than eye muscles in pigmented cats with normal binocular vision The eye muscle abnormalities in Siamese cats were similar to but more pronounced than those seen in pigmented cats reared monocular lid suture at an early age (Lennerstrand & Hanson 1979)

Siamese cats lack binocular vision (Packwood & Gordon 1975) probably as a result of a functional adaptation to a congenital aberration of the visual pathways Part of the optic fibers from the temporal retina in the Siamese cats become misrouted and cross to terminate in the contralateral geniculate body in normal cats these fibers reach the ipsilateral geniculate body (Guillery et al 1974) Some Siamese cats have convergent squint but many have apparently straight eyes However squint was not correlated to the amount of functional adaptation in the visual cortex (Shatz 1977) The type of adaptation was either a suppression of the input from the ipsilateral eye (Hubel & Wiesel 1971) or a suppression of both the inflow from ipsilateral eye and from the temporal retina of the contralateral eye causing binasal visual field defects (Kaas & Guillery 1974 Guillery et al 1974)

In addition Siamese cats showed deficits of contrast sensitivity and a lower visual resolution than normal cats (Blake & Antoinetti 1976) which may be related to a retinal dysfunction due to albinism and lack of pigment in the retina

Some defects of oculomotor functions have been reported in albinos including man Spontaneous nystagmus often occurs from birth (Duke Elder 1963) Albino rabbits showed abnormal optokinetic responses (Collewijn et al 1978) These signs of instability in the oculomotor control system might be related to the abnormalities of the visual pathways as suggested by the latter authors However it is also possible that they were due to the eye muscle abnormalities discovered in this study particularly the lowered fatigue resistance

The eye muscle changes in Siamese cats might represent either a genetically determined alteration of eye muscle development or perhaps more likely muscular adaptations to the congenital defects of binocular function and the lowered visual resolution The eye motor system for binocular vision probably employs mainly the slow contracting and fatigue resistant fiber components found in eye muscles (Lennerstrand 1975 Lennerstrand & Hanson 1978b) In animals lacking binocular vision like the Siamese cats the demand for slow fusion vergence movements might be much reduced and this might lead to a retardation in slow component development It has been suggested that similar mechanisms might induce the lowered fatigue resistance of eye muscles in normally pigmented cats with impaired binocular functions (Lennerstrand & Hanson 1979) Further the fast fiber components seem under-developed in Siamese cats The visual resolution is low in these cats (Blake & Antoinetti 1976) and the movement detection poor (Simoni & Sprague 1976) This might result in a disuse of the saccadic system for fast refixation movements which could then account for the reduced speed of eye muscle contraction in Siamese cats It is also possible that the poor visual resolution curtailed the ability to maintain steady fixation and that due to the decreased demand for tonic fixational activity the slow eye muscle fiber system is affected This would add to the defect that might be caused by the loss of binocular vision and explain why fatigue resistance of eye muscles in Siamese cats is still lower than that of pigmented cats with binocular defects but good visual resolution at least in one eye

Thus both pigmented cats with aquired binocular impairment due to monocular visual deprivation and Siamese cats with a congenital defect of binocular vision showed marked changes of eye muscle function in comparison with the eye muscle properties of normal cats Morphological and histochemical studies are in progress on the muscles examined physiologically They indicate that the functional changes can be correlated with differences in the size of all types of muscle fibers and in the density of capillaries

Acknowledgment

The kindness of Dr Paul Bach y Rita San Francisco ... t use his laboratory facilities is greatly appreciated This study was supported ... Karolinska institutets fonder and NIH Program Project Grant EY-00 ... a)

Referen

Bach y Rita P (1971) Neurophysiology of eye ... y Rita P Collins C C & Hyde J Eds *The control of eye movement* pp ... New York

Berman N & Cynader M (1979) Compariso ... nization of the superior colliculus in Siamese and normal cats *J* ... 383

Blake R & Antoinetti D N (1977) Abnorm ... Siamese cat *Science 197* 109–110

Chino Y M Shansky M S & Hamasaki ... es of X and Y type retinal ganglion cells in Siamese cats *Brain R* ...

Collewijn H Winterson B J & Dubois M F ... netic eye movements in albino rabbits Inversion in anterior visual fiel ... 1353

Cool S J & Crawford M L J (1972) ... ding in striate cortex units of Siamese cats *Vision Res 12* 1809–1814

Duke Elder S (1963) *System of ophthal* ... *abnormal devel pment* part 2 Congenital deformities Mosby St Louis

Guillery R W (1969) An abnormal retinogeniculate projection in Siamese cats *Brain Res 14* 739–741

Guillery R W & Kaas J H (1971) A study of normal and congenitally abnormal retinogeniculate projections in cat *J* ... *Neu* ... *143* 73–100

Guillery R W Casagrande V A & Oberdorfer M D (1974) Congenitally abnormal vision in Siamese cats *Nature 252* 195–199

Hubel D H & Wiesel T N (1971) Aberrant visual projections in the Siamese cat *J Physiol (Lond) 218* 33–62

Hanson J & Lennerstrand G (1977) Contractile and histochemical properties of the inferior oblique muscle in the rat and in the cat *Acta Ophthal (Kbh) 55* 88–102

Kaas J H & Guillery R W (1973) The transfer of abnormal visual field representations from the dorsal lateral geniculate nucleus to the visual cortex in Siamese cat *Brain Res 59* 61–95

Kalil R E Jhaveri S R & Richards W (1971) Anomalous retinal pathways in the Siamese cat An inadequate substrate for normal binocular vision *Science 174* 30 –305

Lane R H Kaas J H & Allman J M (1974) Visuotopic organization of the superior colliculus in normal and Siamese cats *Brain Res 70* 413–430

Lennerstrand G (1975) Motor units in eye muscles In Lennerstrand G & Bach y Rita P Eds *Basic mechanisms of ocular motility and their clinical implications* pp 119–143 Pergamon Press Oxford

Lennerstrand G & Hanson J (1978) The postnatal development of the inferior oblique muscle of the cat I Isometric twitch and tetanic properties *Acta phys* ... *103* 139–143

Lennerstrand G & Hanson J (1978b) The postnatal development of the inferior oblique muscle of the cat II Effects of repetitive stimulation on isometric tension responses *Acta physiol scand 103* 144–153

Table I

Indications and results of pars plana vitrectomy in 100 consecutive cases from 97 patients
For definition of positive and negative result see text

	Total No of eyes	Results		No of patients	Average age (years)
		Positive	Negative		
Diabetic vitreous haemorrhage	37	26 (70%)	11 (30%)	35	51 2 (26–79)
Retinal detachment (vitreous haemorrhage secondary membranes vitreous retraction)	28	11 (39%)	17 (61%)	28	59 1 (7–80)
Vitreous haemorrhage due to various causes (branch vein occlusion central vein occlusion arterial hypertension disciform macular degeneration [illegible] haemorrhage)	17	16 (94%)	1 (6%)	16	60 2 (37–71)
Vitreous haemorrhage due to trauma (perforating injuries contusions and intraocular foreign bodies)	13	6 ([illegible])	7 ([illegible])	13	31 (11–[illegible])
Anterior segment vitrectomy		1	1		[illegible] ([illegible]–71)
Total No of patients				97	
Total No of eyes	100	[illegible]	37		
[illegible] age [illegible]					[illegible] (7–[illegible])

Material and Methods

The material consists of 100 eyes from 97 patients with an average age of 53 1 years (7–80) Average follow up time was 14 2 months (1–38) Indications for vitrectomy and the number of patients in the different groups are shown in Table I

Vitrectomies were performed with the Vitreous Stripper (Kløu 1977a) The stripper is non rotating and cuts in an axial fashion The first 40 cases were performed with the aid of the indirect ophthalmoscope but this method was abandoned as soon as an operation microscope (OPMI 6 Zeiss) was available since the microscope is easier and safer to use The slit lamp mounted on the microscope has been the illumination source All equipment used including corneal contact lenses has recently been described in detail by Kløu (1977a)

Results

Table I shows the results A positive result was obtained in cases with a preoperative cloudy vitreous and a postoperative clear vitreous space (vitreous haemorrhages) In eyes with retinal detachment the majority of the vitrectomies were technical successes but it was not always possible to reattach the retina A positive result in these eyes means reattached retina In the anterior segment group a positive result indicates that the aim of the operation was obtained The change in visual acuity is illustrated in Fig 1 Visual acuity was improved in 55% unchanged in 24% and reduced in 21% on the latest examination in the follow up period

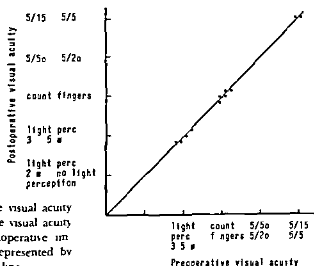


Fig 1
Correlation of preoperative visual acuity (abscissa) with postoperative visual acuity (ordinate) Eyes with postoperative improved visual acuity are represented by dots above oblique line

Table II

Summary of complications and important pre and postoperative factors in 100 consecutive cases of pars plana vitrectomy In brackets the percentage of each column The total in each column exceeds the number in this column because one eye may be represented in more than one category Numbers with – sign represent the failures in each group

	Diabetic vitreous haemorrhage	Retinal detach	Vitreous haemorrhage var caus	Vitreous haemorrhage Trauma	Anterior segment vitrect	Total (%)
Total	37	28	17	13	5	100
Rebleeding, two or three vitrect	8 (22%) 4–	1 (4%) 1–		2 (15%) 1–		11 6–
Retinal tears		3 (11%) 1–		2 (15%) 2–		5 3–
Postop retinal detachment	1 (3%) 1–		1 (6%) 1–	5 (38%) 3–	1 1–	8 6–
Postop haemorrhagic glaucoma	7 (19%) 7–					7 [illegible]
Postop glaucoma contr on medic	3 (8%) 0–					3 0–
Ern... leat during/after vitrect	[illegible]		1 [illegible]	3 [illegible]		[illegible]
[illegible]	[illegible] 1–					[illegible]
[illegible]	[illegible] 1–					[illegible]

Le s I I R	(5 f) 0–	1 (1 f) 1–		1 (8 A) 0–		1 1–
Lens t y during vitrect my	3 (8 t) 0–	1 (1%) 1–		3 (1A) 1–		7 2–
Preop lens/cataract extraction	13 (35 t) 3–	10 (30) 6–			1 0–	27 9–
Postop cataract extraction	4 (11%) 0–	1 (1%) 1–				5 1–
Membrane or cat sec extr during vitrect		6 (21%) 0–				6 6–
Preop photocoagulation	14 (38%) 4–		1 (6%) 0–			15 4–
Postop photocoagulation	3 (8%) 0–	1 (1%) 0–	6 (3 %) 0–			10 0–
Perforating injury prior to vitrect		6 (21) –		7 (4) 3–		13 8–
Previous or present intrabulbar foreign body		3 (11) 3–		4 (31%) 3–		7 6–

A summary of factors of importance pre and postoperatively are listed in Table II The percentages in table and text are calculated to make the groups more comparable

Diabetic vitreous haemorrhage Preoperative visual acuity was less than finger counting 1 meter in all eyes Twenty two of 37 eyes (59%) had visual improvement of varying degree after vitrectomy Vitrectomy had to be repeated in eight eyes due to rebleeding Postoperative haemorrhagic glaucoma developed in seven eyes and led to enucleation in two eyes One eye ended amaurotic because of postoperative endophthalmitis In two eyes where the corneal epithelium was removed during vitrectomy very slow healing took place

Lens opacities were frequent in this group Lens extraction prior to vitrectomy for optical reasons lensectomy during vitrectomy and postoperative lens extraction due to increasing cataract made a total of 20 eyes (54%) aphakic Fourteen eyes had been treated with photocoagulation prior to vitrectomy and 10 of these eyes ended with a positive result

Retinal detachment Vitrectomy was performed because of vitreous opacities (11 eyes) pupillary membranes (6 eyes) and vitreous retinal traction (9 eyes) Retinal detachment was secondary to intracapsular cataract extraction with vitreous loss in seven eyes two eyes had uncomplicated extracapsular extractions and in one eye the lens had been removed because of subluxation after ocular contusion Accidental lens injury happened in one eye during vitrectomy leading to lensectomy Accidental retinal tears occurred in three eyes Retinal detachment surgery after vitrectomy was performed on all eyes except one which was considered inoperable due to massive periretinal retraction (MPR)

Vitreous haemorrhage of various causes The underlying cause of vitreous haemorrhage was central vein occlusion (1 eye) branch vein occlusion (8 eyes) pathologic coagulation mechanism (1 eye) arterial hypertension (2 eyes) disciform macular degeneration (2 eyes) and raised intracranial pressure during subarachnoidal haemorrhage (3 eyes) The interval from thrombosis to vitrectomy varied from 1–21 years (average 9 7 years) and non resorbing haemorrhage had in these eyes been present from 2 months – 15 years (average 22 4 months) One eye with disciform macular degeneration was melanoma suspect and the eye was enucleated on suspicion when the fundus was seen during vitrectomy The histology showed disciform macular degeneration and vitreous bleeding (prep No 1386 7.) One eye developed retinal detachment and was lost due to MPR

Vitreous haemorrhage caused by trauma The underlying cause was ocular contusion (2 eyes) perforating injuries (7 eyes) and intraocular non magnetic foreign bodies (4 eyes) One foreign body was removed by forceps three could not be removed and the eyes were enucleated to avoid the risk of sympathetic ophthalmia Three of seven eyes with perforating injuries were not successful due to retinal detachment and MPR In two eyes retinal detachment developed two months after vitrectomy both ended successfully after retinal detachment surgery Two eyes ended as failures due to retinal tears produced during vitrectomy

Anterior segment Corneal decompensation due to vitreous touching the corneal endothelium induced vitrectomy in four eyes One eye had phacolytic glaucoma, and lens remnants and vitreous were removed These eyes were technical successes but one eye developed a postoperative retinal detachment and retinal surgery was not successful.

Discussion

Vitrectomy gave a positive result in 63% and visual improvement in 55% of our first 100 cases Michels & Ryan (1975) found visual improvement in 64% and Peyman et al (1976) in 68% of their first 100 cases Results on 400 cases are reported (Peyman er al 1978) and results are also published on diabetic eyes (Mandelcorn et al 1976 Michels 1978 Blankenship & Machemer 1978) and on the management of traumatic cases (Hutton et al 1976 Benson & Machemer 1976 Conway & Michels 1978) Complications are reported by Machemer (1972) Kløti (1977b) Tardif & Schepens (1977) Puhlhorn et al (1977) Perry et al (1978)

Complications of diabetes is the most frequent indication for vitrectomy Even if complications to vitrectomy are frequent in this group visual improvement is the result in a high percentage (59% present material 60 8% Michels & Ryan 1975 8% Peyman et al 1976 53% Mandelcorn et al 1976 66% Peyman et al 1978 5% Michels 1978)

In the diabetic group lens extractions were performed for optical reasons but also in order to remove the barrier between the vitreous cavity and the filtration system. Blood in the vitreous cavity is in this way more easily absorbed This view is supported by the result in the present series where 85% of the aphakic eyes (17 out of 20) ended with a positive result compared to 53% of the phakic eyes (9 out of 17)

Corneal complications occurred in two eyes both diabetic Peyman et al (1978) found corneal oedema and striae in 38% in the early postoperative period and as late complication in 7% Perry et al (1978) found that corneal changes developed in 3% Diabetic patients have a poor adhesion of the corneal epithelium (Mandelcorn et al. 1976) and the greatest risk for postoperative corneal problems Many

surgeons do routinely remove the corneal epithelium during vitrectomy. In the present series this has not been performed except in the two diabetic eyes, and the corneas stayed clear during the operation. The experience from this series of eyes does therefore not support the view that it is necessary to remove the epithelium. On the contrary this may induce postoperative corneal complications.

As a total retinal tears occurred in 5% in the present material. Figures vary from 37% (Michels & Ryan 1975) to 2% (Peyman et al. 1978). In eyes with complicated retinal detachment Peyman et al. (1978) had a success rate of 49%. 39% of the eyes in the present series ended reattached. All eyes were however considered to be rather hopeless without vitrectomy and the result must be seen in view of these circumstances.

46% of our traumatic cases ended with visual acuity improved compared to ... in Benson & Machemer's (1976) series, 64% by Hutton et al. (1976) and ... in Conway & Michels (1978) series. The best results are obtained where vitrectomy is performed within 1–2 weeks after the injury.

Ora dialysis (Machemer 1972, 6.6%) and fibrous ingrowth from the sclerotomy opening (Tardif et al. 1977, 11.2%) have not been seen in the present material. The pilot tube for the Stripper is considered important in the prevention of these complications.

References

Benson W. E. & Machemer R. (1976) Severe perforating injuries treated with pars plana vitrectomy. *Amer. J. Ophthal. 81*, 728–732.

Blankenship G. W. & Machemer R. (1978) Pars plana vitrectomy for the management of severe diabetic retinopathy. An analysis of results five years following surgery. *Trans. AAOO 85*, 560–583.

Conway B. P. & Michels R. G. (1978) Vitrectomy techniques in the management of severe penetrating ocular injuries. *Ophth. AAOO 85*, 560–583.

Hutton W. L., Snyder W. B. & Vaiser A. (1976) Vitrectomy in the treatment of ocular perforating injuries. *Amer. J. Ophthal. 81*, 733–739.

Kasner D., Miller G. R., Taylor W. H., Sever R. J. & Norton E. W. D. (1968) Surgical treatment of amyloidosis of the vitreous. *Trans. amer. Acad. Ophthal. Otolaryng.* ...

Klöti R. (1977a) Vitrectomy via the pars plana ciliaris with the vitreous stripper. Instrumentation and technique. In: *Symposium on retinal diseases*. Transactions of the New Orleans Academy of Ophthalmology, pp. 32–40. The C. V. Mosby Company, St. Louis.

Klöti R. (1977b) Prevention of complications during vitrectomy. In: *Symposium on retinal diseases*. Transactions of the New Orleans Academy of Ophthalmology, pp. ... The C. V. Mosby Company, St. Louis.

Klöti R. (1977c) What is the present status of pars plana vitrectomy in Zurich? In: *Mod. Probl. Ophthal.* Ed. Streiff E. B., vol. 18, pp. 187–194. S. Karger, Basel.

Machemer R., Buettner H., Norton E. W. D. & Parel J. M. (1971) Vitrectomy: A pars plana approach. *Trans. amer. Acad. Ophthal. Otolaryng. 75*, 813–820.

Machemer R., Parel J. M. & Norton E. W. D. (19..) Vitrectomy: A pars plana approach ...

Technical improvements and further results *Tran. a ne Ac id Ophthal Ot le ryng* 76 469–466

Machemer R (1979) A new concept for vitreous surgery 2 Surgical technique and complications *Amer J Ophth l 74* 1022–1033

Mandelcorn M S Blankenship G & Machemer R (1976) Pars plana vitrectomy for the management of severe diabetic retinopathy *Amer J Ophthal 81* 561–570

Michels R C & Ryan S J (1975) Results and complications of 100 consecutive cases of pars plana vitrectomy *Amer J Ophthal 80* 24–29

Michels R G (1978) Vitrectomy for complications of diabetic retinopathy *Arch Ophth l (Chicago) 96* 237–246

Perry H D Foulks G N Thoft R A & Tolentino F I (1978) Corneal complications after closed vitrectomy through the pars plana *Arch Ophthal (Chicago) 96* 1401–1403

Peyman G A Huamonte F U & Goldberg M F (1976) One hundred consecutive pars plana vitrectomies using the vitrophage *Amer J Ophthal 81* 263–271

Peyman G A Huamonte F U Goldberg M F Sanders D R Nagpal K C & Raichand M (1978) Four hundred consecutive pars plana vitrectomies with the vitrophage *Arch Ophth l (Chicago) 96* 45–50

Puhlhorn G Teichmann K D & Teichmann I (1977) Intraocular fibrous proliferation as an incisional complication in pars plana vitrectomy *Amer J Ophthal 83* 810–814

Tardif Y M *Schepens C. L. & Tolentino F I (1977) Vitreous surgery XIV Complications* from sclerotomy in 89 consecutive cases *Arch Ophthal (Chicago) 95* 229–234

Tardif Y M & Schepens C L (1977) Closed vitreous surgery XV Fibrovascular ingrowth from the pars plana sclerotomy *Arch Ophthal (Chicago) 95* 235–239

Author's address

Per Syrdalen M D University Eye Department Rikshospitalet Oslo 1 Norway

Department of Ophthalmology (Head Birgitta Zetterström-Karpe), Huddinge University Hospital Karolinska Institutet Stockholm

ULTRASONIC MEASUREMENTS OF THE EYE IN THE NEWBORN INFANT

BY

SVEN BLOMDAHL

The right eyes of 28 newborn infants have been examined retinoscopically and the anterior chamber depth the lens thickness and the length of the vitreous were measured by ultrasound In 15 of the infants keratometry was performed using the Javal Schiotz keratometer The total axial length of the eye was found to be longer the heavier the weight of the baby whereas the lens thickness showed no correlation to the total axial length or to the weight of the baby In only 1/3 of the babies was the corneal astigmatism less than 0.5 D A significant correlation ($P < 0.01$) was found between the total axial length of the eye and the length of the corneal radius

Key words corneal radius – astigmatism – lens thickness – ultrasound – infants – axial length

Ultrasound biometry has recently been used for determining the axial length of the eye in the newborn child as well as the depth of the anterior chamber thickness and the length of the vitreous body (Gernet 1964 Luyckx 1966 Grignolo & Rivara 1968 Larsen 1971) A survey of their values is presented in Table I

Previously the axial length of the eye in the newborn infant had to be measured on cadavers (von Jaeger 1861 von Pflugk 1909 Sorsby & Sheridan 1960)

The aim of the present study has been to investigate the possible interrelationships between the weight of the newborn baby the axial length of the eye the lens thickness the corneal radius and the refraction To this aim the axial depth of the anterior chamber lens thickness and vitreous length have been measured by ultrasound in newborn babies Retinoscopy has been performed and in some cases also keratometry using the Javal Schiotz keratometer

Received December 4 1978

Table I
Earlier determinations of the axial values of the eye in infants

	Larsen		Gernet		Luyckx		Grignolo
	F	M	F	M	F	M	F + M
ant chamber (mm)	2 39	2 37	2 9	2 9	2 5	2 6	-
lens (mm)	3 99	3 93	3 4	3 4	3 6	3 7	-
vitreous (mm)	10 22	10 48	-	-	10 8	10 8	-
tot ax length (mm)	-	-	17 2	17 1	17 7	17 5	17 02
refraction (D)	-	-	+2 6	+3 0	+2 2	+2 6	+0 52
infants examined (n)	37	43	15	21	25	27	18
eyes examined (n)	74	86	29	41	50	54	36
cykloplegic	-	-	Atropin		Cyklogyl		Tropamide

Material and Methods

Twenty-eight healthy fullterm infants 14 girls and 14 boys have been examined Their ages ranged from 1–4 days and their weights from 2310 to 4790 g The eyes to be examined were made cykloplegic by 2 drops of 1% cyklopentolate hydrochloride (Cyklogyl®) instilled twice in the conjunctival sac 45 min later the examination started After local anesthesia with 0 2% oxibuprocaine chloride (Novesin®) a speculum not causing any pressure upon the bulb was inserted to keep the lids apart. The infants were held in an upright position by an assistant Their heads were put on the chin and forehead rest of the Javal Schiotz keratometer The examiner could easily see the reflexes on the cornea and repeated examinations in different meridians were performed In 15 babies everyone quite peaceful the reflexes could be seen on the central cornea and 3–5 repeated examinations showed unanimous low measuring values In 13 babies it was impossible to centralize the reflexes on the cornea With the baby lying down the horisontal corneal diameter was measured using a caliper

Retinoscopy was performed in two axes and finally the ultrasonic biometry could take place This was done with an A-scan apparatus the Kretztechnik 7200 MA and with a transducer frequency of 8 MHz This instrument is equipped with an electronic scale and a built in quarts oscillator which makes calibration possible previous to each examination A contactglass similar to that described by Jansson (1963) and Pallin (1969) was used with an outer opening of 12 5 mm large enough not to press on the cornea during the examination as the corneal diameters were all less than 11 mm The distance between the transducer and the cornea was 15 mm and this part of the contactglass was filled with saline easily refilled (if needed) The baby was lying down during the examination with the head kept steady by an assistant. When the echogram on the screen showed maximal amplitudes from the

reflecting surfaces a polaroid picture was taken. The distance in microsec could then be converted into millimetres according to the vel-ocities given by Jansson (1963) 1532 m/sec in the vitreous and aqueous and 1641 m/sec in the lens at 37°C. Both eyes were measured by ultrasound in 10 of the infants in order to find out if there were any measurable differences between the right and the left eye.

Results

Retinoscopy The range of the retinoscopic readings was from +1.0 D to +5.5 D with an average of +3.6 D. The girls had an average of +3.9 D and the average of the boys was +3.4 D. Sixteen of the 28 infants showed no astigmatism, nine had an astigmatism with the rule and three infants had an astigmatism with oblique axes.

Table II

The retinoscopic findings in two axes, values of the corneal radies in two axes and the corneal power in diopters of the corneas in two axes (calculated from the corneal radius)

infant nr	retinoscopy(D)	corneal radius (mm)	corneal power(D)
1	3.0; 3.0	6.4; 6.5	49.3; 48.8
2	2.0; +4.0	6.5; 7.05	[illegible]
3	1.0; 1.0	6.5; 6.5	[illegible]
4	3.5; 3.5	7.75; .25	[illegible]
5	3.5; 3.5	7; 7	[illegible]
6	X +4.0; 3.0	X 6.9; 5.7	[illegible]
7	3.0; 5.0	6.4; 6.6	53.0; 51.8
8	+4.0; +4.0	7.4; 7.3	[illegible]
9	X +4.5; +2.0	X 7.75; 0	[illegible]
10	3.0; 3.0	X 7.4; 1	[illegible]
11	3.0; +4.0	7; 7	[illegible]
12	X +4.0; 3.0	X 6.6; 6.5	X 51.5; 52.5
13	5.0; 5.0	X 7.2; 7	X 47.0; 45.5
14	3.0; +4.0	7.1; 7	[illegible]
15	3.0; +4.0	6.3; 6.1	52.3; 49

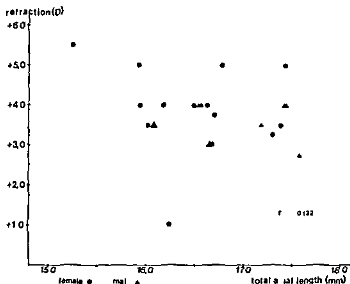


Fig 1
The refractive value plotted against the total axial length of the eye in 28 infants

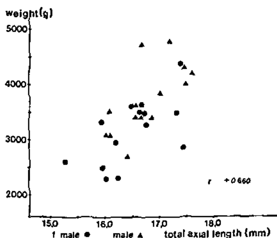


Fig 2
The weight of 28 infants plotted against the total axial length of the eye

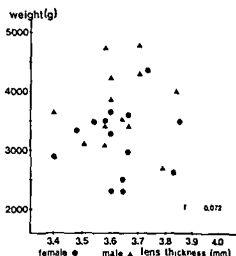


Fig 3

The weight of 28 infants plotted against the total axial length of the lens

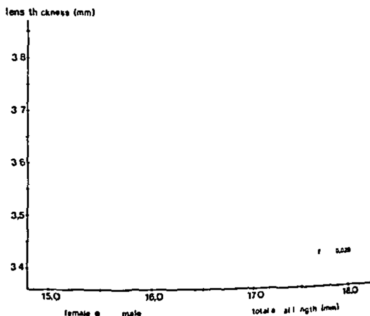


Fig 4

The lens thickness plotted against the total axial length of the eye in 8 infants

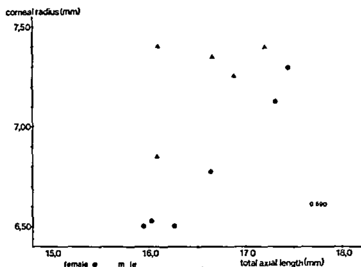


Fig 5
The corneal radius plotted against the total axial length of the eye in 15 infants

The highest astigmatism was 4 D with +6 D in the horisontal and +2 D in the vertical meridian

Ultrasound measurements The depth of the anterior chamber in the 28 children ranged from 2 4 to 2 9 mm The average value was 2 6 mm The central thickness of the cornea was included in these readings

The lens thickness values ranged from 3 4 to 3 9 mm with an average of 3 6 mm The axial vitreous length ranged from 8 9 to 11 2 mm with an average of 10 4 mm The total axial length ranged from 15 3 to 17 6 mm with an average of 16 6 mm The mean value of the boys was 16 7 mm and 16 5 mm of the girls In the ten babies where both eyes were measured by ultrasound the difference of the axial lengths never exceeded 0 2 mm

Keratometry In the 15 babies examined with keratometry the range of the corneal radius was from 6 4 to 7 4 mm with an average of 7 0 mm In five of the babies there was no astigmatism to be found and five babies had an astigmatism with the rule ranging from 1–4 D Five babies showed an oblique astigmatism ranging from 1–2 D Table II shows the values of the retinoscopic figures of the corneal curvature and of the corneal power in the same eye In two of the infants with oblique corneal astigmatism this had not been registered by retinoscopy

Corneal diameter The values of the corneal diameter in the horisontal plane varied from 9 0 to 10 5 mm with an average of 9 8 mm

Correlations Fig 1 shows the association between the refraction and the total axial length of the eye The correlation coefficient is −0 132 and there is no significant correlation between the degree of hypermetropia and the axial length of the eye ($P > 0$ 1) in this group

Fig 2 is a graph of the association between the weight of the baby and the total axial length of the eye The correlation coefficient is 0 660 a value that differs from zero ($P < 0$ 001) showing that the heavier child has a longer eye

Fig 3 shows the relation between the weight of the baby and the lens thickness The correlation coefficient for these parameters is 0 072 a value quite close to zero ($P > 0$ 1) and there is no correlation

Fig 4 shows the association between the lens thickness and the total axial length of the eye Again the correlation coefficient is close to zero (0 0[illegible]9) and these parameters are not significantly correlated ($P > 0$ 1)

In Fig 5 the corneal radius is plotted against the total axial length of the eye in 15 infants The value of the corneal curvature radius is given as the average value of the main meridians The correlation coefficient of the parameters is 0 6[illegible] which significantly differs from zero ($P < 0$ 01) This suggests that the longer the total axial length of the eye the longer the corneal radius i e the flatter the cornea

Discussion

The refractive values of new borns found by other investigators mostly lie between +2 D to +3 D (Duke Elder 1969) In the 28 babies of this study the values varied from +1 0 D to +5 5 D and the average value of +3 6 D is somewhat higher than that normally found

Luyckx (1966) and Larsen (1971) using a contact glass between the eye and the transducer found the anterior chamber to be about 2 4 and 2 6 mm The central thickness of the cornea was included in these values Gernet (1964) found a value of 2 9 mm of the anterior chamber (still including the central corneal thickness) This value however can not be compared to the value found by the former authors because Gernet did not use a contact glass between the transducer and the eye and had to reckon with a pressure on the cornea in his calculations

The mean values of the lens thickness recorded from other ultrasonic studies vary from 3 4 mm to 4 0 mm and the values of the vitreous length from 10 9 mm to 10 8 mm (Table I) The oculometric mean values of the present study i e all in accordance with these figures

The average of the total axial length is 16 6 mm slightly less than the values obtained by Gernet Luyckx and Grignolo but close to the average value 16 [illegible] mm from Larsen s studies

Luyckx and Gernet found no relationship between the refraction and the axial length of the eye neither could this be found in the present study This is rather unique keeping in mind that refraction and axial length are so strongly correlated in all other age-classes The finding implies that in the smaller eye the refractive power of the cornea and – or the lens must be stronger than in the longer eye

In emmetropic eyes of adults Sorsby et al (1957) found a good correlation between the axial length and the corneal power Fledelius (1976) found a significant correlation between these parameters in children aged about ten years This study shows a significant correlation between the total axial length of the eye and the corneal power and the longer the total length of the eye the smaller is the corneal refractive power Thus the cornea compensates for the high degree of hyperopia which otherwise would be expected Changes in lens power might also be compensatory but the present investigation shows no correlation between the lens thickness on one side and refraction or axial lengh on the other

Keratometry performed on stillborn infants in the early 20th century showed a corneal radius of about 7 0 mm (de Vries 1901 von Pflugk 1909) In two living infants Gernet (1964) found a radius of 6 4 mm and 7 7 mm using a Zeiss ophthalmometer Grignolo & Rivara (1968) measured the corneal curvature in premature newborns fullterm newborns and children to the age of ten They used the Javal ophthalmometer and *when this was not possible the measurements were* carried out by juxtaposition of concave stencils representing arcs of circles with known curvature radies They found the corneal power to be +54 3 D in fullterm newborns a value that equivalates with a radius of about 6 3 mm All considered the mean value 7 0 mm of the corneal radius from the present study seems to be close to the mean value of earlier investigators

There seem to be no measurements of the astigmatism of the cornea carried out in living newborn infants Marin Amat (1956) expected most corneas to be spherical This assumption was made on the fact that in children of the age of three 50% had corneal astigmatism and at the age of seven 90% had corneal astigmatism They therefore found it probable that in newborns the astigmatism approximately was zero The present investigation does not favour this theory 66% of the fifteen babies having astigmatic corneas It must be pointed out however that the material is limited

References

Duke Elder S (1970) *Text book of Ophthalmology* V 229 Kimpton London

Fledelius H (1976) Prematurity and the eye Ophthalmic 10-year follow up of children of low and normal birth weight. *Acta ophthal (Kbh)* Suppl 128

Gernet H (1964) Achsenlange und Refraktion lebender Augen von Neugeborenen *Alb v Graefes Arch ophthal 166* 530–536

Grignolo A & Rivara A (1968) Biometry of the human eye from the sixth month of pregnancy to the tenth year of life (measurements of the axial length, retinoscopic refraction total refraction corneal and lens refraction) In Vanysek J ed *Diagnostica ultrasonica in ophthalmologica* II Symposium Internationale de Diagnostica Ultra ... Ophthalmologia 1968 Universita J E Purkyne Brno Czechoslovakia (Opuscula Ophthalmologica Acta Facultatis Medicae Universitatis Brunensis No 35 251–57

von Jaeger J (1861) *Über die Einstellungen des dioptrischen Apparates im menschlichen* ... pp 10–12 L W Siedel u Sohn Wien und Masson Paris

Jansson F (1963) Measurement of intraocular distances by ultrasound and comparison between optical and ultrasonic determinations of the depth of the anterior chamber *Acta ophthal (Kbh.) 41* 25–61

Larsen J S (1971) The sagittal growth of the eye I Ultrasonic measurement of the depth of the anterior chamber from birth to puberty *Acta ophthal (Kbh.) 49* 239–262

Larsen J S (1971) The sagittal growth of the eye II Ultrasonic measurement of the axial diameter of the lens and the anterior segment from birth to puberty *Acta ophthal (Kbh.) 49* 427–440

Larsen J S (1971) The sagittal growth of the eye III Ultrasonic measurement of the posterior segment (axial length of the vitreous) from birth to puberty *Acta ophthal (Kbh.) 49* 441–453

Luyckx J (1966) Mesure des composantes optiques de l aeil du nouveaune par echographie ultrasonique *Arch Ophtal (Paris) 26* 159–170

Marin Amat M (1956) Les variations physiologiques de la courbure de la cornee pendant la vie Leur importance et transcendance dans la refraction oculaire *Bull Soc Belge Ophtal 113* 251–293

Pallin O (1969) The influence of the axial length of the eye on the size of the recorded B potential in the clinical single flash electroretinogram *Acta ophthal (Kbh.)* Suppl 101

von Pflugk A (1909) Die Fixierung der Wirbeltierlinsen insbesondere der Linse des neugeborenen Menschen *Klin Mbl Augenheilk 47* I 1–14

Sorsby A Benjamin B Davey J B & Tanner J M (1957) Emmetropia and its aberrations A study in the correlation of the optical components of the eye *Med Res C unc Spec Rep Ser No 293* London

Sorsby A & Sheridan M (1960) The eye at birth Measurements of the principal diameters in forty-eight cadavers *J Anat (London) 94* 192–197

de Vries W M (1901) Über das Säuglingsauge *Ned T Geneesk I*

Author s address

Dr Sven Blomdahl Huddinge University Hospital
Department of Ophthalmology S-141 86 Huddinge Sweden

University Eye Department (Head: Thore Lie Thomassen) and the Institute of Pathology, Electron Microscope Laboratory (Head: [illegible]) Rikshospitalet, University of Oslo, Norway

VILLOUS PROCESSES FROM THE INNER SEGMENTS OF CONES IN THE HUMAN MACULA LUTEA

BY

MARTIN DAVANGER and AMUND RINGVOLD

The cones of the human macula lutea were examined by scanning electron microscopy. It was found that each inner segment had about [illegible] thread like processes which projected from the cell surface. They were arranged in a longitudinal straight line along the photoreceptor and were directed away from or towards the fovea centralis. The villous processes may be analogous with the lateral fins of the inner segments of certain vertebrates. They perhaps bridge the space between neighbouring photoreceptors and establish contact and interaction between them.

Key words: retina – macula lutea – cones – inner segments

During the fresh specimen dissection of the posterior part of a normal eye ball the retina frequently becomes detached. The two surfaces created, the outer segments of the photoreceptors and the villous apical surface of the pigment epithelium, present themselves for scanning electron microscopy (SEM). However, in some specimens the adherence between the two layers may be stronger. Factors influencing this adherence have been studied and among other things it has been shown that the adherence is stronger *in vivo* than *in vitro* (Zauberman et al. 1972, Zauberman & Guillebon 1972). Part of this adherence may be created by leaf like processes of the pigment epithelium which ensheath the cone outer segments (Hogan et al. 1974, Steinberg & Wood 1974, Steinberg et al. 1977). Clinical experience with retinal detachment indicates that the adherence is stronger in the macular region than in the fundus in general.

Received March [illegible], 1979

Grignolo A & Rivara A (1968) Biometry of the human eye from the sixth month of pregnancy to the tenth year of life (measurements of the axial length, retinoscopic refraction, total refraction, corneal and lens refraction) In Vanysek J ed Diagnostica ultrasonica in ophthalmologica II Symposium Internationale de Diagnostica Ultrasonica in Ophthalmologia 1968 Universita J E Purkyne Brno Czechoslovakia (Opuscula Ophthalmologica Acta Facultatis Medicae Universitatis Brunensis No 35 251–257

von Jaeger J (1861) Über die Einstellungen des dioptrischen Apparates im menschlichen Auge pp 10–12 L W Siedel u Sohn Wien und Masson Paris

Jansson F (1963) Measurement of intraocular distances by ultrasound and comparison between optical and ultrasonic determinations of the depth of the anterior chamber Acta ophthal (Kbh) 41 25–61

Larsen J S (1971) The sagittal growth of the eye I Ultrasonic measurement of the depth of the anterior chamber from birth to puberty Acta ophthal (Kbh) 49 239–262

Larsen J S (1971) The sagittal growth of the eye II Ultrasonic measurement of the axial diameter of the lens and the anterior segment from birth to puberty Acta ophthal (Kbh) 49 427–440

Larsen J S (1971) The sagittal growth of the eye III Ultrasonic measurement of the posterior segment (axial length of the vitreous) from birth to puberty Acta ophthal (Kbh) 49 441–453

Luyckx J (1966) Mesure des composantes optiques de l'oeil du nouveauné par échographie ultrasonique Arch Ophtal (Paris) 26 159–170

Marin Amat M (1956) Les variations physiologiques de la courbure de la cornée pendant la vie Leur importance et transcendance dans la refraction oculaire Bull Soc Belge Ophtal 113 251–293

Pallin O (1969) The influence of the axial length of the eye on the size of the recorded B potential in the clinical single flash electroretinogram Acta ophthal (Kbh) Suppl 101

von Pflugk A (1909) Die Fixierung der Wirbeltierlinsen insbesondere der Linse des neugeborenen Menschen Klin Mbl Augenheilk 47 I 1–14

Sorsby A Benjamin B Davey J B & Tanner J M (1957) Emmetropia and its aberrations A study in the correlation of the optical components of the eye Med Res Counc Spec Rep Ser No 293 London

Sorsby A & Sheridan M (1960) The eye at birth Measurements of the principal diameters in forty-eight cadavers J Anat (London) 94 192–197

de Vries W M (1901) Über das Säuglingsauge Ned T Geneesk I

Author's address

Dr Sven Blomdahl Huddinge University Hospital
Department of Ophthalmology S-14186 Huddinge Sweden

the specimens were dehydrated in graded acetone solutions and dried by the critical point method in fluid CO_2 (Sorvall Critical point drying system) The dried specimens were mounted on specimen holders and coated with a thin layer of carbon and gold palladium in a Edward s vacuum coating unit Scanning electron microscopy was performed with a Jeol JSM 50 SEM

Results

In the specimen from the neuro retina it was found that in large parts of the parafoveal area most of the outer segments of the cones had been torn from the inner segments at the connecting cilium This could be confirmed by the examination of the specimen containing the pigment epithelium In the fovea itself the inner segments had also been removed during the preparation (Fig 1)



Fig 2

Cone inner segments from the parafoveal region displaced by the outer segments being removed during the preparation Numerous thread like processes project from the cell surface On each photoreceptor they are arranged in a longitudinal straight line and they are directed away from or towards the fovea SEM × 5600 Bar = 5 µm



Fig 3
Parafoveal cone inner segments with processes probably cellular villi Arr w p process which seem to bridge between two neighbouring phot recept
Bar = 1 µm

The inner segments appeared as slightly conical elemer outer end tapered gradually off into a thin extension whic the cilium A few remaining outer segments were fou During the preparation the inner segments had bee running parallel forming a sharp angle with the retina' 1–2 µm apart separated from each other by an empty

An unexpected observation was made when the in area were studied with higher magnification (

rocesses measuring up to 2 μm in length protruded about perpendicularly from ie cell surfaces Their diameter w is slightly less than 0 1 μm remarkably constant oth along the single process and from one process to the other They were situated long one straight line from near the cilium and extending usually as far as the ner segment could be seen The distance between the processes was rather niform about 1/3 μm Typically one photoreceptor had about 15 processes

The processes were directed either towards the fovea or away from it the few ueptions were connected with cells which probably had been twisted during the reparation The cell surface seemed to go directly into the surface of the processes ith no discontinuity Most of the processes ended in the intercellular spaces erangement caused by the processing made it difficult to determine with certainty hether the processes *in vivo* had formed bridges between the cells A search for milar processes outside the parafoveal area has been negative

Discussion

he appearance of the single process and the pattern they form together indicate at they represent real histological structures Although their nature cannot be lly determined by SEM alone we suggest that they are cellular villi and to our iowledge similar findings have not been described before In the following the ructures on which we are reporting will be called the lateral processes of the inner gments

It should be noted that three other kinds of cellular extensions or villi are present this region of the human eye (Fig 4) 1) Villous processes extend from the apical rface of the pigment epithelial cells to surround the photoreceptor s outer gment 2) Villi forming the so-called fibrillar basket project from the Muller cells to the intercellular space between the inner part of the inner segment 3) The ner segments themselves have a number of cytoplasmic extensions the so-called lycal processes (Cohen 1961) projecting outwards from the outer end of the ner segments and surrounding the basal quarter of the outer segments The teral processes on which we are reporting may be misinterpreted in transmission lectron microscopical studies as being profiles of one of the cellular extensions nentioned The nature of the lateral processes and their regular distribution is est recognized by scanning electron microscopy

The occurrence in other species of lateral processes of the inner segments or imilar structures remains to be determined The lateral processes may perhaps be analogous with the lateral fins of the inner segments of some lower vertebrates fishes reptiles birds) (Dunn 1973) These processes are radial fin like extensions of the plasma membrane extending laterally from the body of the inner segment

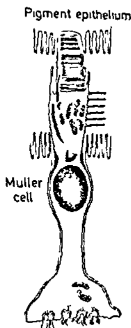


Fig 4

Schematic drawing of a cone with adjacent pigment epithelial cell and Muller cells. The villous processes from these cells are emphasized 1 Villous processes of the pigment epithelium 2 The fibrillar basket villi of the Muller cells 3 The calycal processes of the inner segment surrounding the basal quarter of the outer segment 4 The lateral processes of the inner segment

and imparting a gear like shape to a cross section of the visual cell. The lateral processes described in the present work may perhaps represent vestiges of the lateral fin remaining only in the form of these processes

Nothing decisive can be said about the function of the lateral processes of the inner segments The following possibilities may be considered

1) Other cytoplasmic extensions from the inner segments (the calycal processes (Fig 4) and the lateral fins mentioned above) and also the fibrillar basket extensions of the Muller cells (Fig 4) have been presumed to play a role in the uptake of nutrient material from the extracellular space (Dunn 1966). A similar function may be ascribed to the lateral processes

2) It has been asserted that the intercellular matrix between the photoreceptors and the pigment epithelium serves as an adhesive between these two cell layers (Zimmerman & Eastham 1959) All processes projecting from these cells into the matrix will promote this effect

3) Neighbouring photoreceptors are separated by a space measuring 1–2 µm Up to now no anatomical contact has been demonstrated between human photoreceptors outside the external limiting membrane Although it is difficult to determine with certainty whether the lateral processes have formed bridges between the photoreceptors *in vivo* this may have been the case

Extensive interactions between photoreceptors in toads have been found by physiological experiments and evidence has been presented indicating that the basis for this interaction is the presence of gap junctions between the lateral fins of adjacent photoreceptors (Fain et al 1976)

On this background it may be presumed that the physiological role of the lateral processes of the inner segments is to establish contact and interaction between neighbouring photoreceptors The regular distribution and radial direction relative to the fovea centralis of the lateral processes may be considered on the basis of this concept of their function

As said above the villous processes undoubtedly represent real anatomical structures However as indicated by the conical configuration of the inner segments (Figs 2 and 3) the separation of the retina from the subjacent pigment epithelium by tearing is obviously a rather rough procedure which probably leads to some form changes of the cells In the further studies of these structures therefore a different preparation technique will be used

References

Cohen A I (1961) The fine structure of the extrafo eal receptors of the Rhesus monkey *E p Eye Res 1* 128–136

Dunn R. F (1966) Studies on the retina of the gecko *le x vari g tu* I The visual cell classification *J Ultrastruct Res 16* 651–671

Dunn R F (1973) The ultrastructure of the ertebrate retina In Friedmann I Ed *The ultrastructure f sensory organ* pp 153–265 North Holland Publ Co American Elsevier Publ Co Amsterdam London New York

Fain G L Gold C H & Dowling J E (19 6) Receptor coupling in the toad retina *C ld Spring Harbor Symposia of Qu antitatr e Bi logy* Vol XL The Synapse pp 547–561

Hogan M J Wood I & Steinberg R H (1974) Phagocytoses by p gment epithelium of human retinal cones *Natur 252* 305–307

Steinberg R H & Wood I (1974) Pigment epithelial cell ensheathment of cone outer segments in the retina of the domestic cat *Proc oy Soc lond 187* 461–478

Steinberg R H Wood I & Hogan M J (1977) Pigment epithelial ensheathment and phagocytosis of extrafoveal cones in human retina *Philos Tran (B) 277* 459–471

Zauberman H Cuillebon H de & Holly F J (19 ?) Retinal traction *in vi o* Biophysical aspects *In t Ophthal 11* 46–55

Zauberman H & Guillebon H de (19 2) Retinal traction *n vivo* and postmortem *A ch Ophthal (Chi ag) 87* 549–5 4

Zimmermann L. E. & Eastham A. B. (1959) Acid mucopolysaccharide in the retinal pigment epithelium and visual cell layer of the developing mouse eye. Am. J. Ophthal. 47, 488–498

Author's address

Martin Davanger, University Eye Department
Rikshospitalet, Oslo 1, Norway

ACTA OPHTHALMOLOGICA VOL. 57 1979

Department of Histology (Head: B. Hellman)[1] and
Department of Ophthalmology (Head: L. Berggren)[2]
University of Uppsala, Uppsala, Sweden

MORPHOLOGY AND ENZYME ACTIVITIES OF THE RETINAL CAPILLARIES IN STREPTOZOTOCIN DIABETIC MICE

BY

A. ÅGREN[1], G. REHN[1] and P. NAESER[2]

Male lean mice belonging to the obese hyperglycemic strain were made diabetic by intravenous injection of streptozotocin. The retinal capillary bed freed by trypsin digestion was studied with regard to morphology and the activity of some enzymes. There was a significant increase in the ratio between the endothelial and mural cells which was interpreted as indicating mural pericyte disappearance. The activities of adenylate kinase, aspartate aminotransferase and hydroxyacyl CoA dehydrogenase in the retinal vessels of the diabetic animals were significantly higher than in vessels from the control animals. No differences were found in the activities of glucose-6-phosphate dehydrogenase, glutathione reductase and phosphofructokinase between the two animal groups.
It is suggested that these results reflect early morphological and metabolic changes of the retinal vessels preceding the well known clinical picture of diabetic retinopathy.

Key words: retina – diabetic retinopathy – capillary metabolism – capillary enzyme activities – mice.

Many animal models have been used for experimental studies in diabetic retinopathy. Recently mice with the obese hyperglycemic syndrome which are characterized by marked hyperglycemia and hyperinsulinemia have been studied for possible retinal changes (Naeser & Ågren 1978). No obvious changes in the morphology of the capillary bed were seen. However, the activities of the enzymes adenylate kinase, aspartate aminotransferase and hydroxyacyl CoA dehydrogenase were higher in the obese mice than in the lean non-diabetic controls.

Received March 2, 1979.

The aim of the present investigation was to study the capillary bed in experimental diabetes with low insulin values and to compare the results with those [illegible] the obese hyperglycemic mice For this purpose mice with diabetes induced by streptozotocin were selected

Materials and Methods

In all 127 male lean mice belonging to the obese hyperglycemic strain which has been bred at the Department of Histology Uppsala Sweden since [illegible] (Westman 1968) were used The mice were kept in plastic cages and allowed free access to pelleted food and water (Naeser 1973) At the age of 2 months diabetes was induced by a single injection of streptozotocin (120 mg kg body weight) in the tail vein The streptozotocin was kindly placed to our disposal by Dr W E D[illegible] Upjohn Co Kalamazoo USA Two months after the induction of diabetes the animals were killed and the eyes were rapidly removed and placed in [illegible] formaldehyde solution for 2 h Then the eyes were further processed for morpho*logical and enzymatic determinations as described previously (Naeser & Å[illegible] 1978)* At the time of killing serum glucose was determined from all animals by a glucose oxidase method (Hjelm & deVerdier 1963) For statistical evaluation Student s *t* test was used (Snedecor 1967)

Results

Altogether 77 mice were given streptozotocin injections Fifty non injected lean litter mates served as controls At the time of death the serum glucose was determined Fifty five mice with serum glucose values above 15 mmol l (300 mg per 100 ml) were used The remaining mice had glucose values below 15 mmol l and were not considered diabetic The mean serum glucose concentration of the diabetic animals was 31 1 ± 1 3 mmol/l and differed significantly from the serum glucose of the control animals (8 7 ± 0 2 mmol l $P < 0\,001$)

The ratio of endothelial cells to mural cells was estimated on glass-microscope slide mounted preparations of the retinal vessels In the streptozotocin diabetic mice the ratio was significantly higher (4 82±0 17 n = 8) than in the control animals (3 13±0 02 n = 11 P <0 001) Mesodermal intercapillary bridges were present in all the preparations In the preparations obtained from the streptozotocin diabetic mice the bridges occasionally showed elongated dark nuclei similar to those of [illegible] mural cell nuclei There were no areas showing acellularity or degeneration

The results of the *enzymic activities are given in* Table I It can be seen that [illegible]

Table I

Enzyme activities of the retinal capillaries in streptozotocin diabetic mice

Enzyme	Streptozotocin injected	Non injected	Probability
Adenylate kinase EC 2 7 4 3	185 ± 6 (n = 13)	85 ± 4 (n = 10)	$P < 0\,001$
Aspartate aminotransferase EC 2 6 1 1	47 ± 2 (n = 10)	20 ± 2 (n = 11)	$P < 0\,001$
Glucose-6-phosphate dehydrogenase EC 1 1 1 49	1 0 ± 0 2 (n = 4)	0 9 ± 0 1 (n = 4)	NS
Glutathione reductase EC 1 6 4 2	0 51 ± 0 07 (n = 8)	0 46 ± 0 06 (n = 8)	NS
Hydroxyacyl-CoA dehydrogenase EC 1 1 1 35	2 2 ± 0 1 (n = 14)	1 6 ± 0 1 (n = 11)	$P < 0\,001$
Phosphofructokinase EC 2 7 1 11	4 0 ± 0 1 (n = 9)	4 3 ± 0 1 (n = 11)	NS

All values are expressed as mmol kg dry weight and hour and represent mean values ± SEM Number of animals are given within parentheses The experimental animals were lean mice from the obese hyperglycemic strain

activities for adenylate kinase aspartate aminotransferase and hydroxyacyl-CoA dehydrogenase differ significantly from the controls The activities for glucose-6-phosphate dehydrogenase glutathione reductase and phosphofructo-kinase did not however differ significantly between the groups

Discussion

Decrease in the number of mural pericytes in diabetic retinopathy has been reported in human diabetes (Cogan et al 1961 Kuwabara & Cogan 1963 de Oliveira 1966 Yanoff 1972) and in experimental (Leuenberger et al 1971 1974 Cohen et al 1972 Studer et al 1975 Papachristodoulou et al 1976) and spontaneous animal diabetes (Gepts & Toussaint 1967 Duhault et al 1973 1974) It has been suggested that the loss of these cells is of significance for the development of microaneurysms but so far there is no evidence for this (Cogan & Kuwabara 1967 Yanoff 1969) The increased ratio between endothelial and mural

cells reflects the decrease in the number of pericytes generally seen in [illegible] retinopathy. In contrast, mice with the obese hyperglycemic syndrome having [illegible] hyperglycemia and hyperinsulinemia (Westman 1968) displayed no morph[illegible] changes in the retinal capillaries (Nieser & Ågren 1978). The differences [illegible] pericyte distribution between streptozotocin diabetic mice and obese hyper[illegible] mice raises the question as to what extent the insulin values in [illegible] significance for pericyte degeneration.

The enzyme activities in the retinal capillary bed from the strept[illegible] animals found in the present work are similar to those in the obese hyper[illegible] mice (Nieser & Ågren 1978). This further emphasizes the previous suggestion [illegible] the enzymatic changes may result from the hyperglycemic state. In both [illegible] animal diabetes the differences in enzyme activities may indicate early metab[illegible] changes in the capillaries which are of significance for the later devel[illegible] diabetic retinopathy.

Acknowledgments

Financial support was generously given by the Swedish Diabetes Association, the Swe[illegible] *Medical Research Council (02297 and 00?2?-1 B)*, the Faculty of Med[illegible] University and Carmen and Bertil Regners Foundation.

References

Cohen, A. M., Michaelson, I. C. & Yanko, L. (1972): Retinopathy in rats with disturbed carbohydrate metabolism following a high sucrose diet. *Amer. J. Ophthal.* 73, 863–[illegible]

Cogan, D. G., Toussaint, D. & Kuwabara, T. (1961): Retinal vascular patterns. IV. Diabetic retinopathy. *Arch. Ophthal. (Chicago)* 66, 366–378.

Cogan, D. G. & Kuwabara, T. (1967): The mural cell in perspective. *Arch. Ophthal. (Chicago)* [illegible] 133–139.

Duhault, J., Lebon, F. & Boulanger, M. (1973): *KK mice as a model of microangiopathic diabetes.* 7th Eur. Conf. Microcirculation, Aberdeen 1972, Part 1. Bibl. anat. No. 11 [illegible] 453–458. Karger, Basel.

Duhault, J., Lebon, F., Boulanger, M., Regnault, F. & Kern, F. (19[illegible]) Microa[illegible] diabetique. Etude comparative chez l'homme et l'animal. *Bu[illegible]* [illegible] 11, 167–1[illegible]

Gepts, W. & Toussaint, D. (1967): Spontaneous diabetes in dogs and cats. A pathol[illegible] *Diabetologia* 3, 249–265.

Hjelm, M. & deVerdier, C. H. (1963): A methodological study of the enzymatic determination of glucose in blood. *Scand. J. clin. Lab. Invest.* 15, 415–428.

Kuwabara, T. & Cogan, D. G. (1963): Retinal vascular patterns. VI. Mural cells of the retinal capillaries. *Arch. Ophthal. (Chicago)* 69, 492–502.

Kuwabara, T. & Cogan, D. G. (1963): Conference on microcirculation and diabetic retinopathy (ed. Lyle, D. J.). *Amer. J. Ophthal.* 55, 156–172.

Ieuenberger P Cameron D Strauffacher W Reynold A E & Babel J (1971) Ocular lesions in rats rendered chronically diabetic with streptoz tocin *Oph th I R* 2 189–204

Le enberger P Beruchemin M L & Babel J (19 4) Experimental diabetic retinopathy *A h Ophth il (I ari) 34* 289–309

Naeser P (19 3) Effects of adrenalect my on the obese hyperglycemic syndrome in mice (gene symbol) *Di l t I gia 9* 376–379

Naeser P & Ågren A (19 8) Morpholgy and enzyme activities of the retinal capillaries in mice with the obese hyperglycemic syndrome (gene symbol) *Acta phthal (Kbh) 56* 60–616

Oliveira F de (1966) Pericytes in diabetic retinopathy *Brit J Ophth I 0* 134

Papachristodoulou D Heath H & Kang S S (1976) The development of retinopathy in sucrose fed and streptozotocin diabetic rats *Dia l tol gia 1* 367–374

Snedecor G W & Cochran W G (1967) *Statistical M th ds* Iowa States University Press Ames Iowa

Studer P P Trapp V Siegel F Schmidt F H & Muller A (19) Alterations in retinal capillaries of chronically (12 months) diabetic rats *Di b t l gia 11* 387

We tmar S (1968) Development of the obese hyperglycemic syndrome in mice *D al t l gi 4* 141–149

Yanoff M (1969) Ocular pathology of diabetes mellitus *A er J Ophth l 6* 1–3

Yan ff M (19 9) Histopathogenesis of diabetic retinopathy *Acta h hal 9* – 34

Author s Add ss

Peter Naeser M D Department of Ophthalmology
University Hospital S-75014 Uppsala 14 Sweden

Department of Ophthalmology (Head E Westerlund)
Central Hospital Nykøbing Falster Denmark

TIMOLOL

in maintenance treatment of ocular hypertension and glaucoma

BY

NIELS VESTI NIELSEN and JENS SINDBERG ERIKSEN

The hypotensive effect of Timolol eye drops (0 25 and 0 50%) in maintenance treatment of 64 patients with elevated intraocular pressure has been studied. The patients were treated for a period of mean 13 months In 44 patients mostly comprising ocular hypertensives a significant reduction in IOP (32%) could be maintained with Timolol alone ($P < 0\ 001$)
In 20 patients with a high starting baseline pressure or previous maladjustable glaucoma it was necessary to start combined treatment Pilocarpine epinephrine or acetazolamide appeared to have additive pressure lowering effect to Timolol
A significant correlation was present between pre treatment and Timolol treated intraocular pressures Thus a pre treatment IOP above 25 mmHg may indicate a critically hypotensive effect below an IOP of 20 mmHg with Timolol alone
No significant interference with visual acuity pupillary size blood pressure or pulse rate was noted Existing visual field defects in three patients were slightly aggravated and in four patients with a pathological optic disc visual field loss developed In seven patients transient sensations of dry eyes and rose-bengal staining dots on the cornea developed

Key words Timolol maleate – hypotensive effect – combined treatment – one year result – ocular hypertension – glaucoma – side effects

Previous reports on ocular applied timolol maleate (a β1 and β2 blocking agent) have convincingly demonstrated a potent hypotensive effect on elevated intraocular pressure (Bischoff 1978 Katz et al 1976 Kerty & Horven 1978 Kriegelstein 1978 Nielsen 1978 Radius et al 1978 Ritch et al 1978 Zimmerman & Kaufman 1977a b) At the present these results have been based on short term studies

Received March 2 1979

Compared to other β-adrenergic blocking agents and other antiglaucoma drugs (pilocarpine epinephrine acetazolamide) Timolol has produced an equal or greater reduction in elevated IOP (Bonomi & Steindler 1975 Kriegelstein 1978 Leydecker 1977 Phillips et al 1978 Wetrell 1977) Moreover Timolol has been tolerated well in short term use

The objective of this study has been to investigate the pressure lowering effect and tolerance of Timolol in maintenance treatment of ocular hypertension and glaucoma Further the additive effect of Timolol was studied when combined with pilocarpine epinephrine and/or acetazolamide

Material and Method

The material consisted of 64 out patients (37 females and 27 males) of various ages (mean 66 years range 30 to 85 years) A baseline IOP > 22 mmHg without topically or systemically antiglaucoma treatment for one week was required before entering this study In 31 patients we classified ocular hypertension and in 33 patients glaucoma (optic disc cupping and or visual field loss) Fifty nine patients had open angles three narrow angle glaucoma and two spontaneously partially cured buphthalmos Aphakic glaucoma was present in three patients Forty-eight patients had previously been treated with antiglaucoma drugs with a mean duration of 7 8 years (range 0 5 to 23 years) In the remaining 16 patients recent ocular hypertension or glaucoma were detected Antiglaucoma surgery had previously been performed in 13 patients The main criterias of admitting the previously treated patients to this study were either a maladjustable glaucoma or discomfort of previous treatment Any patients with a history of intraocular inflammations or retinal detachment were excluded from this investigation Further patients with bradycardia (< 55 beats per min) severe heart failure myxoedema bronchial asthma concurrent systemic – adrenergic blocking treatment were omitted from treatment with Timolol Four patients had a mild m turity onset diabetes mellitus

The study was not designed as a blind trial due to long term treatment of the patients However the investigator had no knowledge of previ ous measurements at repeated contr ls or the concentration and dose of Timolol used in the patients

In each patient the treatment was primarily started with Tim l 10 25 administered once daily at 9 p m If the pressure lowering effect of this med cation was inade quate i e > 21 mmHg the patients were treated with Timolol 0 50% once or twice daily at 9 a m and 9 p m In patients where the IOP could not be controlled on Timo l l alone we added pil carpine 2% two or three times daily epinephrine once or twice daily or acetazolamide 00 mg daily

Measurements of the IOP with Goldmann s applanation tonometry were performe l twice at the same time of the day on each contr l During a dose a ljustment peri d f three weeks the IOP was controlled at least once weekl Subsequent contr ls f IOP luring maintenence therapy were performed on the 5th 9th 15th week after the nset of treatment and thereafter with an interval of 12 weeks In addition to pressure measurements ocular

examinations included visual acuity visual field pupillary diameter slitlamp examination including break up time (BUT) of the tear film and rose bengal vital staining corneal sensitivity Schirmer test and ophthalmoscopy Blood pressure and pulse rate were regularly controlled and subjective adverse symptoms if present were recorded

Statistical analyses

The hypotensive effect of Timolol was estimated by means of Wilcoxon's signed rank test (two sample tests)

A correlation between pre treatment baseline pressures and Timolol treated pressures was evaluated by family regression

Results

Intraocular pressure

In 44 patients the IOP was controlled with Timolol alone and in 70 patients combined treatment was instituted Regarding the duration of therapy five subjects had been treated for 6–9 months seven subjects for 9–12 months 37 subjects for 12–15 months and 15 subjects for 15–18 months

In the group of 44 patients treated with Timolol alone a significant reduction of the IOP ($P < 0.01$) could be maintained (Fig 1) The IOP was reduced with mean 32% after one year of treatment This group (mean age 63 years range 33–

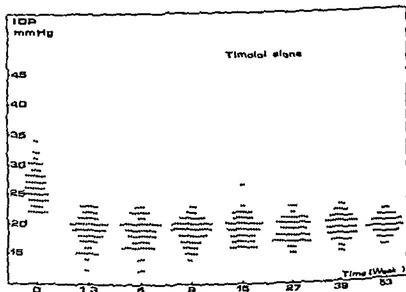


Fig 1

The scattergram illustrates the hypotensive effect of Timolol alone in 44 patients

[illegible] IOI [illegible]

Tr[illegible]	IOI mmHg	Time (we[illegible]k[illegible])							
		0	1–3	[illegible]	9	1[illegible]	27	3[illegible]	53
I	mean	31 0	[illegible]0 8	19 8*	19 1	18 7	18 3	21 5**	18 2
	SD	[illegible] 0	2 2	2 [illegible]	1 8	1 4	3 4	6 5	2 1
	n eyes	6	–	–	–	–	–	–	–
F	mean	[illegible]6 5	21 3*	23 3	[illegible] 5	21 0	21 [illegible]	20 5*	18 5
	SD	2 1	3 9	3 1	3 1	1 8	2 2	1 7	1 2
	n eyes	4	–	–	–	–	–	–	–
E + 1	mean	3[illegible] 5	20 5*	20 [illegible]	21 7	[illegible]0 [illegible]	22 5	19 0	20 3
	SD	7 7	3 5	3 5	[illegible]4	1 3	2 6	1 8	1 7
	n eyes	4	–	–	–	–	–	–	–
A	mean	31 [illegible]	[illegible]3 1**	21 [illegible]*	[illegible]0 6	[illegible]0 [illegible]*	19 6	19 3	19 7
	SD	9 5	3 8	3 7	3 1	2 9	2 6	4 3	3 2
	n eyes	11	–		–	–	–	–	10
A + 1	mean	31 7	[illegible]	[illegible]1 7	[illegible]	[illegible]1 7	[illegible]	20 3	19 [illegible]
	SD	9 4	1 0	[illegible]	[illegible]	[illegible]	3[illegible]	3[illegible]	[illegible] 1
	n eyes			–	–	–	+		–
A + E	mean			[illegible]	[illegible]	[illegible]0 0	[illegible]0	[illegible]0 0	[illegible]0 0
	SD	[illegible]	[illegible]		1 [illegible]	1 4	1 0	1 8	1 5
	n eyes					–	–	–	–

[illegible] indicates the onset of [illegible]

years) consisted of 16 patients with glaucoma and 28 ocular hypertensives Thus 90% (28/31) of the ocular hypertensives contrary to 48% (16/33) of the patients with glaucoma were controlled on Timolol alone No diminuation of the initial pressure lowering effect of Timolol on maintenance therapy was noted in these 44 patients ($P > 0.10$)

In 20 subjects it was necessary to combine Timolol with pilocarpine epinephrine or acetazolamide (Table I) After 1 to 3 weeks of treatment with Timolol 16 patients entered the combined therapy one patient after 15 weeks and 3 patients after 30 weeks This group characteristically comprised patients with glaucoma (85%) previously maladjustable glaucoma high baseline pressures (mean 34 6 mmHg range 23–60 mmHg) and patients of a high age (mean 71 8 years range 58–85 years) The mean reduction in IOP with Timolol alone was 11 7 mmHg (33 8%) and after onset of combined maintenance therapy 14 6 mmHg (41 9%) (Table I)

In Fig 2a predictive estimate of the hypotensive response of Timolol compared with baseline pressure before treatment has been attempted It appears that a starting baseline pressure above 25 mmHg may indicate a critical pressure lowering effect of Timolol alone when a Timolol treated IOP below 20 mmHg is desirable

Antiglaucoma surgery had to be performed in two patients after 20 and 24 weeks on treatment with Timolol In a patient with unmanageable narrow angle glaucoma trabeculectomy was performed and cyclodialysis was performed in a patient with an aphakic glaucoma The latter continued treatment with Timolol

Visual field

Glaucomatous visual field loss was present in 24 patients before treatment with Timolol was instituted After one year on treatment with Timolol a further four patients with an abnormal optic developed a visual field lesion and a slight aggravation of existing visual field defects in three patients

Visual acuity

The pre treatment distance vision was mean 0 80 (range 1 0 to 0 1) No significant changes in visual acuity ($P > 0.10$) were disclosed after 12 months of treatment (mean 0 76 range 1 0–0 01) In nine patients with cataract a slight reduction of visual acuity was noted One patient was operated for cataract In general patients previously treated with miotics obtained a better distance vision on Timolol treatment whereas near vision was disturbed with their former correction

Side effects and adverse reactions

Timolol did not induce changes on pupillary diameter or ... interf... with blood pressure or pulse rate was recorded Investiga...

BUT and rose bengal vital staining have benn reported in a previous paper (Nielsen & Eriksen 1979)

In 10 patients a mild smarting sensation occurred in relation to installation of Timolol Four patients complained of headache for 2 to 3 h following installation Seven patients developed transitory dry eyes and innoxious functional and morphological changes (Nielsen & Eriksen 1979) In none of these patients was treatment with Timolol discontinued After six months of treatment an exudative maculopathy of the left eye occurred in a 70 years male patient with excessive myopia Visual acuity was severely deteriorated a large central scotoma was disclosed

Discontinuance from the study

Four patients were withdrawn from Timolol treatment A 70 years old male patient died from heart failure and pneumonia after 9 months In two patients surgery trabeculectomy (24 weeks) and cataract extraction (36 weeks) was performed and further hypotensive treatment was superfluous The fourth patient did not want to be controlled regularly on Timolol after eight months of treatment

Discussion

The present study demonstrates a powerful and prolonged ocular hypotensive effect of topically used Timolol Thus Timolol seems to be useful in maintenance treatment of elevated IOP Kriegelstein (1978) and Leydecker (1977) have proclaimed that the hypotensive response of Timolol declines after a short time of treatment (tachyphylaxis) just as is noted with atenolol (Brenkman 1978 Wetrell 1977) We did not disclose any significant diminuation of pressure lowering effect in the present material after mean 13 months of treatment However in three cases we noted a subsidence of the hypotensive effect or an aggravation of glaucoma after 29 weeks of treatment It should be emphasized that the occurrence of tachyphylaxis may be difficult to assess in clinical studies

After a dose adjustment period we were able to distinguish two groups of patients with regard to pressure lowering responses of Timolol In general Timolol proved to have the best effect in the group with a majority of ocular hypertensives whereas combined therapy chiefly had to be instituted in cases with previously hard manageable glaucomas and high intraocular pressures In this study as in previous investigations (Bischoff 1978 Kerty & Horven 1978 Nielsen 1978 Radius et al 1978) pilocarpine epinephrine and acetazolamide proved to have an additive effect to Timolol also when used in maintenance treatment

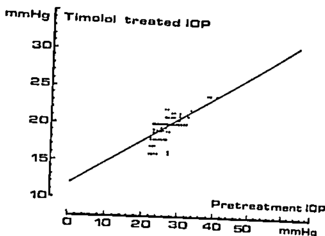


Fig 2

Linear correlation between pre treatment IOP and Timolol treated IOP (y = Bx + A, A 10 7 B = 0 32) in 64 patients (121 eyes) on Timolol alone $P < 0\,01$

Taking the total material into consideration a significant correlation was observed between the pre treatment IOP and the IOP obtained by Timolol (Fig 2) Thus from this relationship it may be possible to predict the level of a Timolol treated IOP from a known pre treatment IOP and to estimate whether combined treatment should be expected Brenkman (1978) has noted an inadequate hypotensive effect of atenolol when pre treatment IOP exceeded 25 mmHg The pressure lowering response of Timolol alone in the present study may be dubious above pre treatment IOP of 28 mmHg

Generally Timolol was tolerated well The dry eyes manifestations were transitory and did not require discontinuance of Timolol therapy (Nielsen & Eriksen 1979) Timolol did not significantly interfere with visual acuity In some of our cases a worsening of existing cataract occurred after one year with Timolol treatment This may obviously appear without hypotensive treatment However Timolol seemingly reduces the aqueous formation (Chiou & Zimmerman 1975 Yablonski et al 1978 Zimmerman et al 1978) Prolonged Timolol induced reduction of aqueous secretion and possible changes of aqueous composition may hypothetically interfere with the metabolism of the lens Treatment with acetazolamide has not induced cataract (Reich 1953) Smith et al (1979) have described a reduction of resting and exercise heart rate during topical treatment with pindolol We have not as other investigators (Bischoff (1978 Kerty & Horven 1978 Kriegelstein 1978 Radius et al 1978 Ritch et al 1978 Zimmerman & Kaufman 1977a b) noted any significant influence of Timolol on blood pressure and pulse rate

At the present Timolol seems to be a useful drug in the future treatment of elevated intraocular pressure

Acknowledgement

Merck Sharpe & Dohme has kindly provided Timolol eyedrops in the present investigation

References

Bischoff P (19 8) Erfahrungen mit Timolol in der Glaucomtherapie *Klin Mbl Augenheilk* 1 3 902-204

Bonomi L & Steindler P (1975) Effect of pindolol on intraocular pressure *Brit J Ophthal* 59 301-303

Brenkman R F (1978) Long term hypotensive effect of atenolol 4% eyedrops *Brit J Ophthal 62* 287-291

Chiou C Y & Zimmerman T J (1975) Ocular hypotensive effects of autonomic drugs *Invest Ophthal 14* 416-417

Katz I M Hubbard W A Getson A J & Gould A L (1976) Intraocular pressure decrease in normal volunteers following Timolol ophthalmic solution *Invest Ophthal 15* 489-492

Kerty E & Horven I (1978) Glaucoma treatment with Timolol *Acta ophthal (Kbh) 56* 05-714

Kriegelstein G K (1978) Die Wirkung von Beta Blockern auf den Augeninnendruck *Klin Mbl Augenheilk 172* 677-685

Kriegelstein G K (1978) Die Wirkung von Metoprololtartrat auf den Augendruck *Klin Mbl Augenheilk 173* 632-637

Leydecker W (1977) Sympathikomimetika und Sympathikolytika in der Glaucomtherapie *Klin Mbl Augenheilk 171* 538-546

Nielsen N V (1978) Timolol Hypotensive effect used alone and in combination for treatment of increased intraocular pressure *Acta ophthal (Kbh) 56* 504-509

Nielsen N V & Eriksen J S (1979) Timolol Transitory manifestations of dry eyes in long-term treatment. *Acta ophthal. (Kbh)* in press

Phillips C I Gore S M & Gunn P M (19 8) Atenolol versus adrenaline eye drops and an evaluation of these two combined *Brit J Ophthal 62* 296-301

Radius R L Gary R D Pollack I P & Langham M E (19 8) Timolol A new drug for management of chronic simple glaucoma *Arch Ophthal (Chicago) 96* 1003-1008

Reich T (1953) Anhydrase activity of bovine lenses *Amer J Ophthal 36* 500

Ruch R Hargett N A & Podos S M (1978) The effect of 1 5% Timolol malleate on intraocular pressure *Acta ophthal (Kbh) 56* 6-10

Smith S E Smith S A Reynolds F & Whitmarsch V B (19 9) Ocular and cardiovascular effects of local and systemic pindolol *Brit J Ophthal 63* 63-66

Wettrell K (1977) Beta adrenoceptor antagonism and intraocular pressure A clinical study on propranolol practolol and atenolol *Acta ophthal (Kbh) Suppl 134*

Yablonski M E Zimmerman T J Waltman S R & Becker B (1978) A fluorophotometric study of the effect of topical Timolol on aqueous humor dynamics *ARVO abst acts 172*

Zimmerman T J & Kaufman H E (1977a) Timolol A B adrenergic blocking agent for the treatment of glaucoma *Arch Ophthal (Chicago) 95* 601-604

Zimmerman T J & Kaufman H E (1977b) Timolol Dose response and duration of action *Arch Ophthal (Chicago) 95* 605-607

Zimmerman T J Harbin R Pett M & Kaufman H E (1977) Timolol and facility of outflow *Invest Ophthal 16* 623-624

Author's address

Niels Vesti Nielsen M D Department of Ophthalmology Central Hospital DK-4800 Nykøbing Falster Denmark

Department of Ophthalmology (Head E Westerlund)
Central Hospital Nykobing Falster Denmark

TREATMENT OF ACUTE OCCLUSION OF THE RETINAL ARTERIES

BY

NIELS VESTI NIELSEN

In five patients with obstruction of the retinal arteries three with emboli the circulation was reestablished by bulbus massage intravenous administered acetazolamide and theophylamine The patients were further treated with salicyclic acid for six months An improvement of vision was obtained and maintained over a 2 year period The basis of the present treatment of occlusive arterial disorders in the retina is discussed

Key words retinal artery occlusion – ocular massage – hypotensive treatment – theophylamine – salicylates

The occurrence of retinal arterial occlusion is presumably not uncommon in elderly people In the study of Karjalainen (1971) the incidence of retinal arterial occlusion among outpatients at hospital was about 1‰ over a period of 11 years. However sufficient information of the real incidence of arterial obstruction in the retina is not available Arterial hypertension and arteriosclerosis is frequently present in patients with occlusive vasculopathy in the retina (Lorentzen 1969 Karjalainen 1971) Degenerative vasoobliteration and emboli of various origin are the major causes of retinal arterial obstruction (Ffytche 1974)

The treatment of arterial occlusion in the retina has previously been characterised with more or less enthusiasm Thus various methods (surgical and medicamental) and in general disappointing results have been reported (Brown & Shields 1979 Ffytche 1974 Kuchle 1977 Magargal & Goldberg 1977 Wise et al 1971)

In this paper an apparently effective management of acute arterial obstruction in the retina will be presented

Presented at the 478 meeting of the Danish Ophthalmological Society April 19 9
Recieved April 4 1979

Material and Methods

n five consecutive cases with obstruction of a branch or central retinal artery an immediate treatment was started in the hospital Temporal arteritis was excluded The patients were treated regardless of the duration of symptoms of arterial cclusion

In all patients X ray of thorax ECG and stethoscopical examination of the chest and the major arterial truncs of the neck were performed Haemoglobin thrombocytes leucocytes ESR serum protein electrophoreses SM 12 autoanalyser programme and serum lipid cholesterol triglyceride were controlled Ocular examination comprised visual acuity visual field slitlamp examination ophthalmoscopy and photography of the fundus

Procedure of treatment

1) *Massage of the bulbus* The eyeball was initially pressed firmly for 15 sec against the bony nasal wall of the orbit while the gaze of the patient was directed downwards The eyeball was then pressed and depressed abrupt y and rhythmically between two fingers with such a force that a deformation of the bulbus could be felt This procedure was performed for 3 to 5 min in three series unless retinal flow had already been reestablished

2) *Ocular hypotensive treatment* All patients were treated with acetazolamede 500 mg intravenously on admission followed by 500 mg perorally daily for 3 months In 4 patients Timolol 0 5% eye drops twice daily was further used in the acute phase

3) *Vasoactive medical treatment* An intravenous theophylamine drop (1 g in 500 ml glucose) was administered for 24 h after the massage of the eyeball On admission a bolus of 200 mg theophylamine intravenously was administered before treatment with the intravenous drop

4) *Platelet inhibition treatment* Salicylic acid tablets 1 gram daily for six months were used

Case Reports

Case 1

October 19 6 a 74 year-old man was hospitalised for reduction of the left eye vision occurring six h before admission Since 1972 he had been blind in the right eye owing to a nontreated retinal artery occlusion Visual acuity was decreased to 6/20 in the affected eye With the right eye he could perceive hand movements at one meter In the lower nasal and temporal quadrant of the central visual of the central visual field the patient recorded a large scotoma in the left eye On ophthalmoscopy an embolus was localized in the upper macular



Fig. 1a and 1b
Fluorescein angiography illustrating the result of treatment in Case 2. Fig. 1a demonstrates embolic obstruction of the retinal arteries. In Fig. 1b the retinal circulation has been restored after treatment.

arteriole with boxcar phenomena distal from the embolus. The retina was pale and oedematous in the upper part of the posterior pole. Intraocular pressure was 14 mmHg in both eyes and no pathological changes were noted in the anterior segment of the eye. The embolus was dislodged by bulbus massage in 15 min. Next day visual acuity was measured < 6/6 and diminuation of scotoma was registered. No somatic pathological findings were present. On follow up examination two years later the ocular status was unchanged.

Case 2

A 72 year old man was admitted 27.07.77 because of blurred vision for four h in the right eye. In two days the patient had several episodes with amaurosis fugax without other symptoms. In 1964 the patient had a severe cranial fracture with optic atrophy of the left eye. Visual acuity was 6/60 in the right and only slight light perception was present in the left eye. Three large paracentral scotomas were registered in the right visual field. Intraocular pressure was 20 mmHg in both eyes. On ophthalmoscopy several arterial emboli were seen in the posterior pole of the right eye. Optic atrophy of the left eye was noted. Fluorescein angiography of the right eye demonstrated obstruction of the retinal arteries to the posterior pole (Fig. 1a). Half an hour after treatment fluorescein angiography showed an improvement of the retinal circulation (Fig. 1b). Next day the visual acuity was improved to 6/6 with 1.0 sph. and the scotomas had disappeared. Physical and laboratory findings were normal. At follow up control in March 1979 no recurrence had appeared.

Case 3

In a 68 year-old woman a sudden reduction in visual acuity of the right eye occurred 15.09.77 without concurrent ocular or somatic symptoms. The duration of this symptom had been three h. Visual acuity was 6/20 in the right and 6/6 in the left eye with + 1.5 sph. on both eyes. On the right eye the patient had developed a paracentral scotoma. Slitlamp examination



F 2a an l_b

The fundus of the patient in C ise ɔ Complete occlu ion of the retin il arteries and diffi e retinal ischemia are present (Fig 2a) After treatment the retin l flow is impr ved (Fig 2b)

and pupillary reactions were normal Intraocular pres ure as 14 mmHg in thr right and 16 mmHg in the left eve In the lower temporal artery of the affected eye an embolus was localized A systolic cardiac murmur was disclo ed The embolus could be removed by bulbus massage but it reappeared four times during the next two days On the third day it disappeared The visual acuity was improved on remo al of the embolus to 6 6 with + 1 ɔ sph and the paracentral scotoma remitted Reexamination in December 1978 showed unchanged ocular findings

Case 4

A 9 year-old woman was primarily admitted 02 08 77 for a total retinal artery obstruction and blindness of the left eve which had been present for two days At that time no effect of treatment occurred Two months later the vision of her right eye deteriorated The time interval before treatment then was three h Vision of the right eye was reduced to light perception in the temporal visual field compared to 6 6 measured 02 08 77 The pupils reacted slightly on light stimulation Intraocular pressure was 20 mmHg bilateral Ophthal moscopy and fluoresscein angiography disclosed nearly complete obstruction of retinal circulation The retinal circulation was improved after bulbus massage for 30 min After two days a visual acuity of 3/60 was measured and 14 days later it was 6/24 The patient suffered from an arteriosclerotic cardiac disease with mitral insufficiency On a reexamination in Juli 78 visual acuity was 6/18 and no visual field lesions could be demonstrated

Case 5

A 7 year-old man was admitted 18 01 79 for total blindness of the right eye This symptom occurred suddenly 19 h before admis ion The patient had no other ocular or somatic complaints One year ago the patient experienced an episode w th amaurosis fugax in the right eye On admission there was no light perception or reaction of the pup l in the right eye Visual acuity of the left eve was 6 6 with −7 0 sph The anterior segment of the eye was

normal on slitlamp examination Intraocular tension was 15 mmHg in the right and 16 mmHg in the left eye Ophthalmoscopy of the right eye disclosed a pallor of the optic disc and complete occlusion of the central artery with diffuse isschemia of the retina (Fig 2a) The left fundus appeared normal After 10 min of bulbus massage the retinal circulation was restituted (Fig 2b) After one week and the following month visual acuity was improved to [illegible] with −3 0 sph In visual field a central scotoma and a slight peripherical constriction were present Physical and laboratory examinations disclosed no abnormalities

Discussion

The results of the present material indicate that active treatment of arterial occlusion in retina may have a favourable effect The use of ocular massage apparently had the most convincing and instantaneous effect on occluded retinal arteries In this series the best visual results 1−2 years after treatment were obtained in 3 cases with emboli localised anterior to the lamina cribosa In none of the patients was a cilioretinal artery present. It is generally assumed that cloudy swelling of the retinal meurones develops within 3 h after complete arterial obstruction (Ffytche 1974) In this material the majority of occlusions must have been incomplete explaining the effect of treatment despite the rather long duration of impaired vision

Physical vasodilatation by massage of the globe a simple method was already introduced in 1882 by Wood White Using this method it is possible to dislodge an atheromatous clot or embolus Further a considerable improvement of the arteriolar volume flow (80−180%) has been demonstrated in experimental animals (Ffytche et al 1974) In the above mentioned cases this method had been performed intensely and repeatedly in each subjects as emergency therapy Thus a rather instantaneous improvement of the retinal circulation was demonstrated with subsequent improvement of vision Vasodilatation induced by paracentesis of the anterior chamber may presumably have the same effect as bulbus massage (Magargal & Coldberg 1977 Brown & Shields 1979) However in animals studies paracentesis has failed to produce more than 20% increase in the retinal volume flow (Ffytche et al 1974) Moreover this method is practically more difficult in handling acute arterial occlusion with repeated treatment and continuous registration of the retinal vascular flow

The use of theophylamine intravenously as an additive treatment in this material is theoretically supported by the fact that this drug especially provokes a vasoconstriction in normal cerebral vessels thereby increasing the flow to the focal diseased areas − inverse intracerebral steal phemomenon (Skinhøj & Paulson 19[illegible]) The vasomotoric tonus of the retinal vessel in many respects is autoregulated by the same mechanism as the cerebral vessels (Wise et al 1971)

The administration of vasodilating drugs apparently may have an autoward effect on the cerebral and possibly the retinal circulation due to a marked vasodilatation of the normal vessels and subsequent decreased flow within the obstructed area – steal phenomenon (Skinhøj & Paulson 1970 Wise et al 1971) However a vasodilatation may be useful when vasospasms predominate especially in young people

Salicylic acid was used with the aim of reducing the risk of a tromboembolic recurrence by inhibition of platelet aggregation (Harrison et al 1971) During the past years evidence has accumulated that antiplatelet treatment may be beneficial in cerebrovascular tromboembolic disease (Didisheim et al 1974)

Only few reports have dealt with the spontaneous course of untreated arterial occlusion in the retina (*Kuchle 1977 Magargal & Goldberg 1977*) *It must be* stressed that controlled prospective blind studies are reuired to assess the significance of active treatment surgical and pharmalogical compared to the results of *mt ea ed arterial obstruction in the retina*

The results of the present series indicate however that ocular massage and intravenous administered acetazolamide a simple treatment could reasonably be *performed in patients with retinal arterial occlusion before admission to hospital*

References

Brown C C & Shields J A (1979) Cilioretinal arteries and retinal arterial occlusion *Arch Ophthal (Chicago)* 97 84–92

Didisheim P Kasmier F J & Fuster V (1974) *Platelet inhibition in management of* trombosis *Tr n Diath Haemorrgag* 32 21–9C

Ffytche T J Bulpitt C J Archer D Kohner E M & Dollery C T (1974) Effect of changes in intraocular pressure on the retinal microcirculation *Brit J Ophth l* 58 514–522

Ffytche T J (1974) A rationalization of treatment of central retinal artery occlusion *T n Ophthal S c* 4 468–478

Harrison M J C Marshall J Meadows J C & Russel R W (1971) Effect of aspirin in amaurosis fugax *Lancet* 2 743–744

Karjalainen K (1971) Occlusion of the central retinal artery and retinal branch arterioles *Acta phth l (kbh) Suppl* 109 1–96

Kuchle H J (1977) Zur Therapie der akuten arteriellen Durchblutungsstörungen von der Netzhaut und Sehnerv *Klin Mbl Augenhe lk* 171 395–40C

Lorentzen S E (1969) Occlusion of the central retinal artery *A ta phth l (Kbh)* 47 690–703

Magargal L E & Goldberg R E (1977) Anterior chamber paracentesis in the management of acute nonarteritic central retinal artery occlusion *Surg Foru* 28 518– 21

Skinhøj E & *Paulson O B (1970) The mechanism of action of aminophylline upon cerebral* vascular disorders *Acta Neurol Scand* 46 129–140

Wise C Dollery C & Henkind P (1971) *The Retinal C r l t n* Harper & R w Publishers *Inc* New York pp *124–126* 314–315

Wood White (1882) *Ophthal Re* 1 49

Author address

Niels Vesti Nielsen M D Department of Ophthalmology
Central Hospital 4800 Nykøbing F lster Denmark

Department of Ophthalmology[1] (Heads: [illegible]) and Department of Oto-rhino-laryngology[2] (Heads: [illegible]), Odense University Hospital, Odense, Denmark

OCULAR LESIONS IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA

BY

INGE VASE[1] and POUL VASE[2]

A regional survey has been carried out in order to establish the prevalence of hereditary haemorrhagic telangiectasia (HHT) (Rendu-Osler-Weber's disease). Forty seven patients fulfilled the proband criteria. No patient was registered due to ocular symptoms.
All of the probands were subjected to ophthalmological examination in order to estimate the distribution of ocular lesions in HHT. Conjunctival lesions were found in 20 cases and an intraocular vascular abnormality in only one case. The possibility of intraocular lesions being a component of the clinical picture of HHT or a sporadic vascular abnormality is discussed.

Key words: hereditary haemorrhagic telangiectasia – Osler's disease – conjunctival lesions – retinal lesions

Hereditary haemorrhagic telangiectasia (HHT) is a rare autosomal dominar hereditary disease with typical vascular abnormalities in respect of histopatholog and localization. The telangiectases are localized to the venules and capillaries in th skin and mucosa. Recurring epistaxis is a predominant trait but bleeding may occ from the conjunctival sac, oral mucosa or gastrointestinal tract.

Several reports have appeared, mainly regarding conjunctival telangiectases sinc the first investigations on ocular lesions in HHT (Chauffard 1896, Weber 1907 The first description of intraocular vascular lesions was given by Gjessing (191 and later by François (1938). There have been few reports on retinal lesions an these are mainly divided into two groups: partly varicous venules and paravascul haemorrhages (Cuendet & Magnenat 1953, Landau et al. 1956, Calmettes et a 1958) and partly arteriovenous aneurisms (Forker & Bean 1963, Davis & Smi

Received May 16, 1979

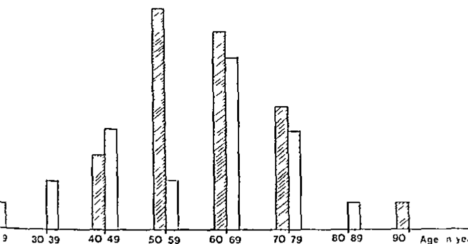


Fig 1
Age and sex di tribution at time of examin ti n

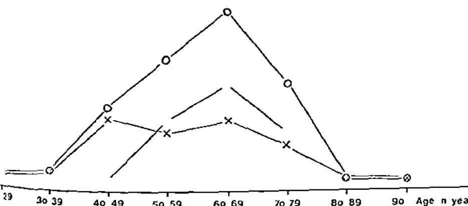


Fig 2
elations between patients with and th out ocular le ions n here I tary h emorrhagi telangiectasia



Fig 3

Typical conjunctival lesions in Osler s disease with clusters of telangiectases

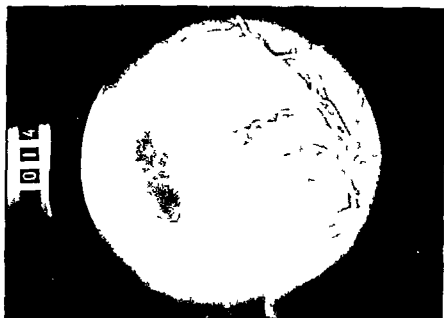
venule was observed during ophthalmoscopy Some tortuosity but no haemorrhage was seen in this area The vascular deformity in the right eye was demonstrated by means of fluorescien angiography

Multiple lesions were found in the conjunctivae in another case with severe affection of the skin and mucous membranes Ophthalmoscopy of this patient showed no signs of intraocular vascular deformities and although fluorescein angiography was carried out no abnormality was observed

Discussion

There is good general agreement in respect of the conjunctival lesions between the findings of the present investigation and reports in the literature as to the pronounced similarity between the conjunctival lesions and the mucosal lesions elsewhere in the system

The intraocular lesions however show a more heterogeneous picture Cessin (191) described retinal paravascular haemorrhages in a 26 year old man suffering from severe anaemia secondary to recurrent epistaxis In 1938 Francois described



F g 4
Fluorescein angiogram showing an arterio-venous shunt between retinal artery and upper temporal venule in the right eye of a 67 year-old woman with Osler s disease

retinal vascular lesions with dilated and tortuous retinal venules in a 74 year-old woman with hypertension Landau et al (1956) found bilateral varix like venous ectases in the retina of two sisters In 1963 Forker & Bean found a unilateral retinal vascular abnormality in a 19-year-old man with tortuosity of the venules simulating a complex arterio-venous aneurysm

Ehlers & Jensen (1973) suggested a mesodermal dysplasia as the common cause of different retinal lesions However the intraocular vascular lesions differ considerably in occurrence and variability from the remaining clinical manifestations of HHT

The present authors however are of the opinion that the retinal lesions are a rare vascular abnormality in a normal population rather than a part of the clinical picture of hereditary haemorrhagic telangiectasia.

References

Calmettes H Deodati H H & Bec H H (1958) Les hemorragies retiniennes dans la maladie de Rendu-Osler B ll Soc Opht al (F a ce 58 482—483

Chauffard M A (1896) Hemophilie avec stigmates teleangiectasiques Soc Med Hop Paris Serie 3 352–358
Cuendet J F & Magnenat P (1953) Symptomatologie oculaire de la maladie de Rendu-Osler Schweiz med Wschr 83 1531–1533
Davis D G & Smith J L (1971) Retinal involvement in hereditary hemorrhagic telangiectasia Arch Ophthal (Chicago) 85 618–623
Ehlers N & Jensen V A (1973) Hereditary central retinal angiopathy Acta ophthal (Kbh) 171–178
Forker E L & Bean W R (1963) Retinal arteriovenous aneurism in hereditary hemorrhagic telangiectasia Arch intern Med 111 778–783
François J (1938) Langiomatose hemorrhagique familiale et ses complications oculaires Arch Ophthal (Paris) 2 425–432
Gjessing E (1915) Telangiectasia hereditaria hæmorrhagica (Osler) Hospitalstidende (Kbh) 1151–1159
Landau J Nelken E & Davis E (1956) Hereditary hæmorrhagic telangiectasia with retinal and conjunctival lesions Lancet 271 230–231
Miles M E (1952) Hereditary hemorrhagic telangiectasia Amer J Ophthal 35 343–346
Pandolfi M & Ehinger B (1978) Conjunctival bleeding in Osler's disease with associated platelet dysfunction A case report. Acta ophthal (Kbh) 56 75–82
Weber F P (1907) Multiple hereditary developmental angiomata of the skin and the mucous membranes associated with recurring hæmorrhages Lancet 2 160–162

Author's address

Inge Vase Department of Ophthalmology
Odense Sygehus DK 5000 Odense Denmark

Department of Ophthalmology[1] Laniado Hospital Netanya and
Soroka Medical Center Faculty of Health Sciences[2]
Ben Gurion University of the Negev Beersheva Israel

CONGENITAL HORIZONTAL GAZE PARALYSIS FACIAL HEMIATROPHY AND SITUS INVERSUS OF THE OPTIC DISC

A case report

BY

LEONARD ROTHKOFF[1] and BENZION BIEDNER[2]

A case of congenital horizontal gaze paralysis in association with facial hemiatrophy and situs inversus of the optic discs is described Except for the presence of deafness on the side of the atrophic face no other neurologic abnormality was present. To our knowledge no similar case has been reported.

Key words gaze paralysis – facial hemiatrophy – situs inversus

Congenital horizontal gaze paralysis has been described in association with facial palsy in some cases of Mobius Syndrome (Hicks 1943) and in a case of Klippel Feil syndrome (Witzel 1958) The purpose of this report is to describe a congenital gaze disturbance with facial hemiatrophy and situs inversus of both optic discs

Case report

A six year-old boy was referred to us with the diagnosis of congenital esotropia. He was born after a normal pregnancy and delivery to unrelated parents There is no family history of ocular abnormalities Psychomotor development has been normal

On examination asymmetry of the face was apparent, with the left side moderately atrophic as compared to the right side (Fig 1) This was confirmed by X rays which showed hypoplasia of the mandible and zygoma on the left side (Fig 2) The left ear was smaller than the right, with a preauricular nodule and atresia of the external meatus (Fig 3) Audiometry revealed moderate hearing loss on the left side due to a conduction defect.

Received March 23 1979



Fig 1
Facial assymetry caused by hemiatrophy of the left side Note smaller ear on this side Eyes appear to be straight in the primay position



Fi 2
Facial X ray showing hyp plasia f the mandible and zygoma on the left side



Fig 3
Preauricular nodule and malformed left ear



Fi 4
Full vertical movement
Top looking up bottom looking d n



Fig 5
Attempted lateral gaze Top looking to the right bottom looking to the left.
Note retraction of the non fixing eye

Since birth on the posterior scalp near the midline a cauliflower like soft nodule measuring 2 × 2 × 2 cm had been present which was originally diagnosed as a meningocele but on re-evaluation at the age of three years was felt to be a dermoid with an intracranial sinus tract On plain skull films no intracranial epidermoid cyst was seen

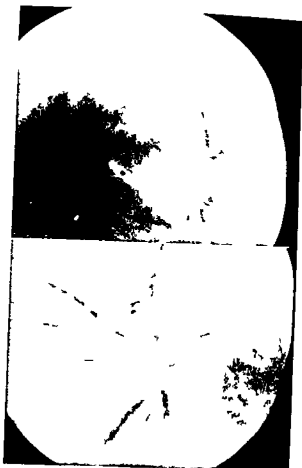
Visual acuity was 6/6 in both eyes On straight ahead gaze the eyes were orthophoric (Fig 1) Vertical movements were normal (Fig 4) However no lateral movement could be elicited Instead attempts at horizontal gaze were replaced by convergence movements (Fig 5) in association with pupillary constriction and moderate retraction of the non fixating eye Opticokinetic response could be obtained vertically but not horizontally Similarly caloric stimulation could not elicit any horizontal ocular movement Convergence occurred normally with no retraction noted upon near point testing Forced ductions were normal

Ophthalmoscopy revealed situs inversus of both discs (Fig 6) The macula and periphery were normal

Neurologic examination was normal with no evidence of facial nerve palsy found The rest of the physical examination showed no abnormality

Discussion

The congenital gaze disturbance described in this case is similar to that previously described (Zweifach *et al* 1969) and is characterized by 1) absence of version on attempted conjugate lateral gaze 2) the use of convergence instead of adduction for lateral viewing 3) retraction of the non fixing convergent eye on attempted lateral



Fi 6
Situs inversus of optic discs Top right eye Bottom left eye

gaze 4) and the presence of normal vertical movements As in our case these findings may be misinterpreted as a congenital esotropia with cross fixation if not for the presence of orthophoria in the primary position and the presence of the near reflex with ocular retraction on attempted lateral gaze

The retraction of the convergent non fixing eye on attempted lateral gaze is similar to the prominent sign in Duane s Syndrome Paradoxical innervation of both horizontal recti has been shown in the retracting eye in such cases (Breinin 1957) and was alluded to in a case such as ours where electromyography was attempted (Zweifach et al 1969) It is noteworthy that narrowing of the palpebral fissures was not present in our case

The presence of vertical movements and convergence in the absence of horizontal gaze even with opticokinetic and caloric testing can be explained by the

separate anatomic centers for these functions Our case would be typical of a lesion in the pons affecting the horizontal gaze centers while sparing the midbrain centers for vertical movement and convergence

Failure of lateral movements of both eyes is usually associated with bilateral facial paralysis in the congenital Mobius Syndrome Despite the presence of facial hemiatrophy no evidence of facial nerve involvement was present The anomalies of the external ear and conduction deafness are typical in this condition (Duke Elder 1964) The etiology of facial hemiatrophy is unknown It has not previously been reported in association with horizontal gaze palsies

Situs inversus of the optic disc is a congenital anomaly whereby the central retinal vessels emerge toward the nasal instead of the temporal side Though often associated with additional ocular defects such as myopia inferior conus (Caccanuse 1954) temporal field defects refraction anomalies and amblyopia (Manor 1974) we could find no report of motor anomalies except for that of exotropia (Fishman et al 1976)

A common pathogenesis for these three anomalies not previously associated is not apparent It is generally accepted that horizontal gaze palsies ar due to a lesion in the pontine centers for lateral gaze (Zweifach et al 1969) Various hypotheses have been presented for the facial hemiatrophy including a lesion of the seventh nerve but none has been proven (Duke Elder 1964) The situs inversus a form of dysversion of the optic nerve is seemingly unrelated to the other anomalies and is not present in other family members The most that can be proposed in a common embryotoxic factor acting early in fetal life

References

Breinin G M (1957) Electromyography – a tool in ocular and neurologic diagnosis II Muscle palsies *Arch Ophthal (Chicago) 57* 165–175

Caccanuse W C (1954) situs inversus of the optic disc *Amer J Ophthal 38* 854–856

Duke Elder S (1964) *Syst m f Ophthalmol gy* vol III part 2 p 1026 London Henry Kimpton

Fishman J Spaier A H & Cohen M M (1976) Familial dextrocardia divergent strabismus and situs inversus of optic disc *Amer J med Sci 271* 225–231

Hicks A M (1943) Congenital paralysis of lateral rotators of eyes with paralysis of muscles of face *Arch Ophthal (Chicago) 30* 38–42

Manor R S (1974) Temporal field defects due to nasal tilting of discs *Ophthalm logi a 168* 69–781

Mitzel S H (1958) Congenital paralysis of conjugate gaze occurrence in a case of Klippel Feil syndrome *A ch Ophthal (Chicago) 59* 463–464

Zweifach P H Walton D S & Brown R H (1969) Isolated congenital horizontal gaze paralysis occurrence of the near reflex and ocular retraction on attempted lateral gaze *A ch Opl thal (Chicago) 81* 345–350

Author s addre s B Biedner M D Soroka Medical Center
P O Box 151 Beersheva Israel

Eye Clinic (Head K Bech)
Frederiksberg Hospital Copenhagen and
Childrens Hospital (Heads F Tersler B Petersen and K F Lderen)
Fuglebakken Copenhagen

CHORIORETINITIS OF THE NEWBORN WITH HERPES SIMPLEX VIRUS TYPE 1

Report of a case

BY

PER REERSTED and BENTE HANSEN

A disseminated herpes simplex virus (HSV) infection involving the central nervous system and accompanied by chorioretinitis in a 3 week-old girl is described The aetiologic diagnosis was established on the basis of virus isolated from skin vesicles and a significant rise in complement fixing antibodies to HSV type 1
The mode of transmission of the virus to the infant apparently was direct contact with an oral lesion in the mother that was present at the time of delivery The patient survived but became blind and microcephalic with severe neurological sequelae
The virus isolated was identified as HSV type 1 which is an infrequent finding in herpetic chorioretinitis of the newborn

Key words chorioretinitis – herpes simplex virus – type 1 – newborn

Since Cogan et al (1964) published a study of retinopathy attributable to HSV in a 3 week-old infant this subject has attracted considerable attention According to Cibis (1975) 14 out of 160 reported cases of neonatal HSV infection had chorioretinitis However none of the typed cases with chorioretinitis were associated with HSV type 1 infection (Nahmias et al 1975)

The present report describes a case of disseminated infection with CNS involvement accompanied by chorioretinitis in a 3 week old girl The virus cultured was identified as HSV type 1

Received April 30 1979

Case Report

A 3 ı eek-old girl was admitted under the diagnosis of malnutrition to Childrens Hospital Fuglebakken Copenhagen on November °6th 1977

The infant was born in the 36th week of gestation weighing °800 g Three days before admission she was noted to be quiet and unresponsive Vesicles were observed on her left cheek and on her chest

A clinically manifest maternal HSV infection was reported at the time of delivery A large vesicle on the upper lip persisted for 2–3 weeks of nursing No evidence of pri r herpetic infections was recorded Two months after delivery the mother developed dendritic keratitis in her left eye She was treated at the Department of Ophthalmology Municipal Hospital of Copenhagen with topical application of iododeoxyurid ne and adenine arabinoside after which the lesion vanished

On admission the infant was lethargic with low body temperature (34 C) Vesicles in clusters were seen in the upper right part of her chest and a few lesions were found on her left cheek There was n involvement of the oral cavity or the eyes On the second day she developed seizures and on the following day the course was stormy with fever seizures difficulties of breathing and vasomotor instability Her breathing had to be assisted mechanically There were s gns of disseminated infection with hepatomegalia and pathological liver parameters A few more vesicles appeared until the eighth day

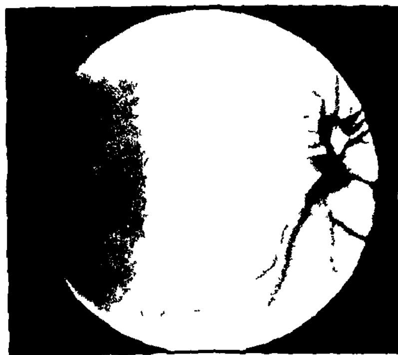


Fig 1
Central scarring (r ght eye)

Five and six months later small vesicles recurred on the left cheek They were treated with iododeoxyuridine ointment Systemic treatment with antiviral preparations was omitted.
The patient is still alive but she is blind and microcephalic with severe neurological sequelae

Ophthalmological examinations Ophthalmoscopy on the fourth day and six weeks after admission was normal A third examination under general anaesthesia at the age of 14 weeks however showed bilateral chorioretinitis in the equatorial and central regions The vitreous was clear and the optic disc seemed without atrophy Centrally the foveola was atrophic with a halo of pigmentation (Fig 1) In the equatorial region circumscript chorioretinal scarring most prominent in the upper parts was seen (Fig 2) The scars were composed of severely hypertrophic pigmentation and white atrophic areas The protrusion was approximately three dioptres The lesions were somewhat more widely distributed in the left eye

Virological studies Viral cultures from the initial skin vesicles revealed HSV They were typed by the mouse hepatitis test (Mogensen et al 1974) as HSV type 1 as

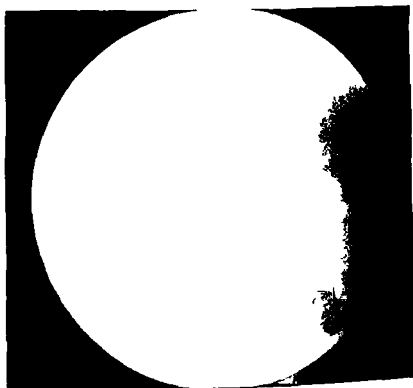


Fig 2
Part of equatorial scarring (right eye)

were cultures from skin lesions appearing seven months later Complement fixing antibody titers to HSV type 1 rose from "negative" on the second day of admission to 1 240 seven weeks later The maternal antibody titer to HSV 3½ months after delivery was 1 60 Scrapings from the cervix uteri six weeks later showed no signs of virus

Additional investigations A lumbar puncture on the day of admission showed no cells or bacteria No bacteria or viruses were cultured IgG was within normal limits IgM and IgA were subnormal EEG was diffusely abnormal Toxoplasma titers of mother and child were negative

Discussion

Herpes simplex virus exists in two closely related antigenic types HSV type 1 is primarily associated with nongenital infection whereas HSV type 2 is associated with genital infection together with occasional infection of the newborn (Nahmias & Roizman 1973)

HSV infection of the newborn is a serious condition with a high fatality rate and severe sequelae are common in survivors Among the sequelae reported those involving the CNS and the eye predominate (Nahmias et al 1975)

Since most cases of neonatal HSV infection are acquired on passage through an infected birth-canal the majority of isolated virus chains (>70%) belong to the genital type of the virus (HSV type 2) whereas the oral type (HSV type 1) accounts for only about 30% The source of the virus in the latter cases may either be genital HSV type 1 maternal infection a non genital maternal infection or a non maternal one (Nahmias et al 1975)

The present history of recognizable maternal HSV lesions on the lips at the time of delivery and in the left cornea a few weeks after delivery is highly suggestive of a maternal infection caused by HSV type 1 as was actually isolated in the infant The onset of symptoms in the reported case falls within the range of the incubation period of neonatal HSV infections from birth to three weeks reported by Nahmias et al (1975) This and the rise in HSV antibody titer from negative on the second day of admission to 1 240 50 days later is strongly indicative of postnatal transmission of the virus from a presumably oral infection in the mother An infection in the mother s genital tract cannot, however be excluded

The retinal lesions observed 2½ months after the initial infection are presumably cicatricial indicating a previous acute chorioretinal infection (Cibis et al 1978)

Nahmias et al (1975) stressed that the number of reported neonatal HSV type 1 infections was now large enough to permit a preliminary view of whether or not the clinical spectrum varies according to HSV type They did not find any striking

differences with the two virus types in 89 cases of HSV type 2 and 40 cases of HSV type 1 infection with the one exception of chorioretinitis having so far been observed only with HSV type 2

The present case seems to be among the first ones to show chorioretinitis in a neonatal HSV type 1 infection indicative of common clinical appearance of the two HSV types in chorioretinitis of the newborn

Addendum

The patient died of her sequelae April 1979 and unfortunately autopsy could not be done

References

Cibis G W (1975) Neonatal herpes simplex retinitis *Albrecht von Graefes Arch. klin. Ophthal.* *196* 39–47

Cibis G W Flynn J T & Davies E B (1978) Herpes simplex retinitis *Arch Ophthal (Chicago)* *96* 299–302

Cogan D G Kuwabara T Young G F & Knox D L (1964) Herpes simplex retinopathy in an infant *Arch Ophthal (Chicago) 72* 641–645

Mogensen S C Teisner B & Andersen H K (1974) Focal necrotic hepatitis in mice as a biological marker for differentiation of herpesvirus hominis type 1 and type 2 *J gen. Virol.* *25* 151–155

Nahmias A J & Roizman B (1973) Infection with herpes simplex viruses 1 and 2 (third of three parts) *New Engl J Med 289* 781–789

Nahmias A J Visintine A M Reimer C B Buono I Del Shore S L & Starr S E (19) Herpes simplex virus infection of the fetus and newborn *Prog Clin. Biol Res.* 3 63–77

Author's address

Per Reersted Eye Department Hvidovre Hospital
Kettegård Alle 30 DK 2650 Hvidovre Denmark

Department of Ophthalmology (Head: David Miller) and
Department of Neurology (Head: Norman Geschwind)
Beth Israel Hospital and Harvard Medical School, Boston, Mass. USA

IN VITRO PRODUCTION OF STEROID CATARACT IN BOVINE LENS

Part I. Measurement of optical changes

BY

DAVID MILLER, M. L. TIJERINA and CHAIM MAYMAN

Fresh calf lenses were incubated in nutritive media to which was added ouabain and dexamethasone phosphate at concentrations of 1×10^{-4} M. At the end of a three day incubation period cortical opacification developed in the control series of lenses as well as those lenses incubated in both ouabain and dexamethasone phosphate. Using a light transmission device which quantitates lens opacification, it was noted that dexamethasone produced a level of cortical opacification significantly greater than that of the control series. Ouabain produced a level of cortical opacification statistically identical to that produced by the dexamethasone. It is suggested that the aforementioned light transmission device is an accurate and reproducible method of quantitating cataractous opacification.

Key words: steroid cataract – in vitro cataract – cataract quantification – ouabain cataract – cataract turbidity

Research on cataracts has been conducted by scientists representing medicine, biochemistry and physics. Collecting and comparing the results of research from these disciplines is important if cross fertilization of ideas is to take place. To this end we feel that standardizing the measurement of the severity of the cataractous process is an important first step in encouraging communicaton between researchers.

In this paper we intend to (a) describe a simple and precise method for measuring experimental cataracts and (b) use the technique in assessing the severity of cataracts produced by ouabain (Bonting et al. 1963, Bonting 1965, Palva &

Received March 27, 1979

measured Preliminary studies showed that light incident to the lens was scattered by all portions of the lens Thus the light received by the photomultiplier probe gathered a representative sample from all portions of the experimental lenses

Results

Table I presents the light transmission data in arbitrary units for each group of differently treated lenses along with a comment on the statistical difference between each series and the control

Table I reveals that both dexamethasone and ouabain induce opacities of the same degree

Fig 2 demonstrates the appearance of typical lenses in the control (C) ouabain (OUA) dexamethasone (DEX) and fresh (F) groups

Both the ouabain and dexamethasone induced opacities were significantly greater than the control lens opacity

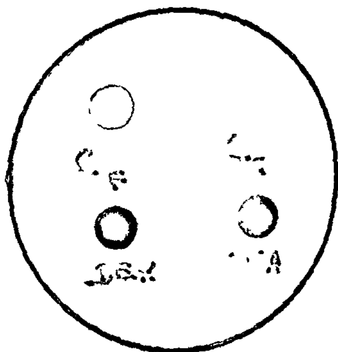


Fig 2

The appearance of calf lenses after 3-day incubation Top left fresh control lens Top right incubated control lens (3 days) Lower left lens incubated in 1×10^{-4} M dexamethasone Lower right lens incubated in 1×10^{-4} M ouabain Whiteness signifies transparency and blackness signifies opacity

In each case the opacity was always located in the lens cortex leaving the lens nucleus clear Using the dissecting microscope set at 30× magnification one could see that the individual lens fibers of the cortex were opaque as was the layer of epithelial cells just under the lens capsule If the fresh lens were left in incubation media plus dexamethasone for two hours small vacuoles were noted to form along the boundary between cortex and nucleus

Thus from a macroscopic point of view the experiment seems to demonstrate the following series of events in a lens incubated in culture medium First there is an accumulation of vacuoles which ultimately coalesce at the border between the cortex and nucleus Then an opacification of the cells and fibers of the cortex develops Finally a peeling off of the opacified cortex takes place leaving a clear nucleus

Discussion

The present study follows the cortical opacification of fresh calf lenses incubated for three days in nutritive media. The process appears to be accelerated by a factor of 7 if either ouabain or dexamethasone at a concentration of 1×10^{-4} M is added to the medium

Since the degree of lens opacification produced by dexamethasone was identical to that produced by ouabain in our series of experiments and since ouabain has been shown to inhibit Na^+ K^+ ATPase in the lens the data suggest that both may act via the same mechanism that of inhibition of Na^+ K^+ ATPase in the lens

We also noted that use of the light transmission apparatus described in the study gave precision to the measurement of cataract opacification far beyond th 1 to 4+ system conventionally used

Acknowledgments

The authors would like to thank Dr Leo T Chylack for his advice in organizing the tudy and his constant counsel throughout the work The authors would also like to thank Profe so George Benedek and Dr Jin Kinoshita for their comments and suggestions during the stud

References

Bettman J W Jung W E & Noyes P O (1964) Potentiating action of prednisone on galactose cataracts in rats *Invest Ophthal 3* p 628

Bonting S L (1965) Na K activated adenosine triphosphatase and active cation transport in the lens In *Symposium on the Lens* Harris J E ed p 347 C V Mosby Comp St Louis

Bonting S L Caravaggio L L & Hawkings N M (1963) Studies on sodium potassium activated adenosine triphosphatase VI Its role in cation transport in the lens of cat calf and rabbit *Arch. Biochem 101* p 47

Kinoshita J (1974) Mechanism initiating cataract formation *Invest Ophthal* 13 p 713
Kinoshita J & Obazawa H (unpublished data)
Mayman C I (1972) Inhibitory effect of dexamethasone on sodium potassium activated adenosine triphosphatase of choroid plexus in cat and rabbit *Fed Proc* 31 p 591 abs
Mayman C I (1974) Developmantal aspects of sodium potassium activated adenosine triphosphatase (Na^+ K^+ ATP ase) in choroid plexus *Neurology (Minneap)* 24 3 0–3 1
Mayman C I Tijerina M L (1976) Effect of dexamethasone on sodium potassium activated adenosine triphosphatase of choroid plexus *J Neurochem* (submitted for publication)
Mayman C I Miller D & Tijerina M L (1979) In vitro production of steroid cataract in bovine lens Part II – Measurement of sodium potassium adenosine triphosphatase activity *Acta ophthal (Kbh)* 57 1107–1116
Miller D Benedek G (1973) *Intraocular Light Scattering* C C Thomas Springfield Ill
Palva M Palkama A (1974) Histochemically demonstrable sodium potassium activated adenosine triphosphatase (Na K ATP ase) activity in the rat lens *Exp Eye Res* 19 p 11
Tarkkanen A Esila R & Liesmaa M (1966) Experimantal cataracts following long-term administration of corticosteroids *Acta ophthal (Kbh)* 44 665–668
Wood D C Contaxis I Sweet D Smith J C Jr & VanDolah J (1967) Response of rabbits to corticosteroids I Influence on growth intraocular pressure and lens transparency *Amer J Ophthal* 63 849–856
Wood D C Sweet D Contaxis L & VanDolah J (1967) Response of rabbits to corticosteroids II Influence of topical therapy on lens aqueous humor serum and urine composition *Amer J Ophthal* 63 849–856

Author's address

David Miller M D Department of Ophthalmology
Beth Israel Hospital 330 Brookline Avenue Boston MA 02215 USA

Department of Neurology (Head Norman Geschwind) and
Department of Ophthalmology (Head David Miller)
Beth Israel Hospital and Harvard Medical School
Boston Massachusetts USA

IN VITRO PRODUCTION OF STEROID CATARACT IN BOVINE LENS

Part II Measurement of sodium potassium adenosine triphosphatase activity

BY

CHAIM I MAYMAN DAVID MILLER and M L. TIJERINA

Fresh calf lenses incubated in nutritive media containing dexamethasone phosphate or ouabain in concentrations ranging from 1×10^{-4} M to 1×10^{-8} M developed cortical opacification and showed significant inhibition of Na^+ K^+ ATPase activity. Over a 3-day incubation period the decrease in Na^+ K^+ ATPase activity correlated well with the observed decrease in light transmission. The degree of enzyme inhibition and decrease in light transmission varied directly with the concentration of dexamethasone phosphate and ouabain with significant changes observed at physiologic and pharmacologic concentrations of these agents. Lenses incubated for 4 days in dexamethasone phosphate or ouabain showed substantial increases in water content as well as an increase in Na^+ and a decrease in K^+ concentration. These data suggest that inhibition of the cation pump may play a significant role in the formation of steroid cataract *in vitro*.

Key words: dexamethasone – inhibition – light transmission – Na^+ K^+ ATPase – ouabain

An extensive clinical experience has shown that cataract formation occurs in some patients receiving long term steroid therapy for conditions such as rheumatoid arthritis, asthma and nephritis (Gullberg & Elman 1973, Loredo et al. 1973, Kennedy 1970, Benatti & Ball 1971, Kristensen 1968). It has been found to occur rather frequently in children, even those on short term and low-dose therapy (Behari & Grossman 1968, Taub 1968, Braver et al. 1966). It has also been shown

Supported in part by grants from The Ridley Foundation, New York, and Harold and Miriam Green Charitable Foundation, Toronto.

Received March 27, 1979

that topical corticosteroid administration in the therapy of ophthalmic disease produces a similar effect The cataract produced under these circumstances is typically posterior subcapsular in location and presents a serious problem to those afflicted in this manner However the mechanism of steroid cataract formation remains obscure In recent years Kinsey Kinoshita and others (Kinsey 19 ; Kinoshita 1974 Iwata & Kinoshita 1971) in studying various types of cataract formation have stressed the importance of cation fluxes across the lens and their relation to the cation pump of which the enzyme Sodium Patassium Adenosine Triphosphatase (Na^+ K^+ ATP ase) is an important component Maintenance of the pump leak balance is considered crucial to the preservation of the viability and transparency of the lens Interference with pump function by the specific inhibitor ouabain (Bonting 1965 Bonting et al 1963) or in the Nakano strain of mice (Iwata & Kinoshita 1971) causes accumulation of water and the formation of a cataract Some authors have shown (Harris & Becker 1965) that steroids exert an effect on cation balance in the lens The mechanism however of the change in cation balance has not been elucidated

Recently Mayman and Tijerina have reported an inhibitory effect of the steroid dexamethasone 21 phosphate on the Na^+ K^+ ATP ase of choroid plexus (Mayman 1972 1974 Mayman & Tijerina 1979) This inhibition is of the same order of magnitude as that of ouabain Postulating that inhibition of this enzyme in the lens and thereby of the pump should produce cataract formation Miller et al (1979) reported in the previous paper cataract formation when calf lenses were incubated for three days in medium containing 1×10^{-4} M dexamethasone 21 phosphate The cataract thus formed was of the same degree and type as that produced by incubation in equimolar concentrations of ouabain

The present communication reports significant inhibition of Na^+ K^+ ATPase in cataractous lenses incubataed in media containing as little as 1×10^{-7} M dexamethasone 21 phosphate The inhibition produced by dexamethasone 21 phosphate is similar to that produced by ouabain In the body of this paper the term dexamethasone refers to the use of dexamethasone 21 phosphate

Material and Methods

Calf eyes obtained from a local abattoir were delivered to the laboratory within one to two hours after the death of the animal The lenses were removed atraumatically from the enucleated globe and homogenized in a glass homogenizer in 0 05 M tris buffer containing 0 05 M EDTA The homogenate was centrifuged at 13000 g for one hour according to the method of Kinoshita (1974) Na^+ K^+ ATP ase activity of the insoluble fraction was measured according to the methods of Samson & Quinn (1967) and Lowry & Lopez (1946) in the presence of varying concentrations of dexamethasone and ouabain

In another series of experiments fresh calf lenses were prepared and incubated individually

dually in medium containing varying concentrations of dexamethasone in a manner described in the foregoing communication (Miller et al 1979) Lenses were also incubated in media containing similar concentrations of ouabain Control incubations of lenses were carried out in identical media containing neither dexamethasone nor ouabain

At intervals during a three-day incubation period the lenses were removed from the medium and light transmission measured in the manner previously described (Miller et al 1979) After measurement of light transmission whole lenses were homogenized in 0 05 M tris buffer pH 7 4 containing 0 05 M EDTA using a teflon coated homogenizer The insoluble fraction was collected by centrifugation of the homogenate at 13000 *g* for 60 min in a Sorvall RC2 B centrifuge The residue was resuspended in 1 0 ml of 0 05 M tris buffer pH 7 4 and used for Na^+ K^+ ATPase assay

The Na^+ K^+ ATPase assay based on the measurement of inorganic phosphate (Pi) liberated by the Na^+ K^+ ATPase activity is carried out in the following manner The complete incubation medium of 1 0 ml contained tris buffer 0 05 M pH 7 4 ATP 5 0 mM $MgCl_2$ 5 0 mM NaCl 100 mM and KCl 20 Mm The reagent medium was equilibrated at 37°C for 5 min and the reaction begun by addition of 25 µl of tissue homogenate Incubations were carried out in a water bath at 37°C for 5 min and the reaction stopped by placing the reaction mixture in ice Aliquots were removed and Pi measured according to the method of Lowry & Lopez (1946) The Na^+ K^+ stimulated ATPase activity was determined by the difference in activity in the presence and absence of Na^+ and K^+ Formation of inorganic phosphate (Pi) was found to be linear with time over this incubation period Appropriate standards and blanks were treated in an identical manner A zero time tissue blank was run by placing tissue in the reagent medium and stopping the reaction immediately

The protein content of the tissue suspensions was determined by the method of Lowry et al (1951)

To asses the water content of lenses after incubation in dexamethasone or ouabain (1×10^{-4} M) for four days lenses were removed from the incubation medium patted dry with filter paper weighed then heated at 120 C until steady weight had been obtained

In a similar series of experiments Na^+ and K^+ content was measured in lenses incubated for three days in dexamethasone or ouabain After incubation lenses previously exposed to each type of medium were pooled and homogenized in a glass homogenizer in ten volumes of water The homogenate was centrifuged and filtered An aliquot of the filtrate was used for Na^+ and K^+ measurements in a flame photometer

Results

Fig 1 shows the Na^+ K^+ ATPase activity of the 13000 *g* insoluble fraction in the presensce of varying concentrations of dexamethasone and ouabain A pharmacologic dose response curve is seen with concentrations ranging from 1×10^{-4} to 1×10^{-8} M It can be seen that dexamethasone inhibits most of the ouabain inhibitable portion of the enzyme At concentrations that are considered to be pharmacologic i e 1×10^{-5} M 60 per cent inhibition occurs with lower levels of inhibition occurring at lower concentrations

When lenses were incubated for three days in media containing either dexamethasone or ouabain 1×10^{-4} M Na^+ K^+ ATPase activity was measured at daily

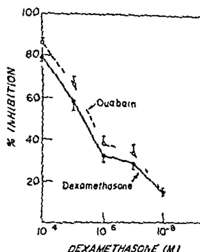


Fig. 1

The inhibitory effect of dexamethasone and ouabain on Na+ K+ ATPase of calf lens. Vertical bars indicate standard error of the mean. The numbers of lenses used in these experiments were as follows

10−4 M−−−−20　　10−5 M−−−−10
10−6 M−−−−10　　10−7 M−−−−10
10−8 M−−−−10

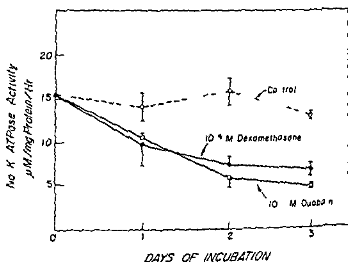


Fig. 2

Changes in Na+ K+ ATPase activity in lenses incubated in dexamethasone or ouabain containing media for 3 days. Vertical bars indicate standard error of the mean. The numbers of lenses used in these experiments were as follows. No. lenses 0 time = 0

Day 1 = C (6)	Dex (4)	Oua (4)
Day 2 = C (6)	Dex (4)	Oua (4)
Day 3 = C (6)	Dex (4)	Oua (4)

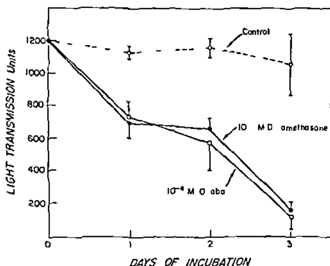


Fig 3

Changes in light transmission through lenses incubated in dexamethasone or ouabain containing media for 3 days Vertical bars indicate standard error of the mean The numbers of lenses used in these experiments were as follows N lenses 0 time = 20

Day 1 = C (6)	Dex (4)	Oua (4)
Day 2 = C (6)	Dex (4)	Oua (4)
Day 3 = C (6)	Dex (4)	Oua (4)

intervals over this period These data were correlated with daily light transmission measurements as described in the previous communication (Miller et al 1979) Figs 2–3 illustrate the changes observed over this period It is clear that control lenses show only slight decrease in light transmission over the three-day incubation period while lenses incubated in dexamethasone or ouabain show a marked decrease in light transmission These changes in light transmission correlate well with a decrease in Na^+ K^+ ATP'ase activity observed over the same period of time There was an initial change in both light transmission and enzyme activity By the second day enzyme activity was 47 and 37 per cent of control in the case of lenses incubated in dexamethasone and ouabain respectively Light transmission was reduced to 57 per cent in ouabain containing media Thus the changes in light transmission and enzyme activity were of similar magnitude over this period of time

At the end of the three-day incubation enzyme activity was 51 per cent of control with dexamethasone and 38 per cent in the case of ouabain Therefore the greatest percentage decrease in enzyme activity occurred in the first 48 h of incubation while the greatest relative decrease in light transmission occurred between 48 and 72 h of incubation

Table I

Light transmission in calf lenses incubated in dexamethasone or ouabain (% of control)

Concentration	Control	Dexamethasone	Ouabain
1×10^{-3} M	100	17 ± 2 (21)	3 ± [illegible]
1×10^{-4} M	100	14 ± 1 (51)	13 ± 1 [illegible]
1×10^{-5} M	100	14 ± 1 (21)	[illegible]
1×10^{-6} M	100	91 ± 2 (18)	[illegible] (18)
1×10^{-7} M	100	67 ± 11 (10)	49 ± 1 (10)

Numbers in brackets represent number of lenses in each experiment

Tables I and II show the effect on light transmission and ATPase activity in lenses incubated for three days in dexamethasone or ouabain at concentrations ranging from 1×10^{-3} to 1×10^{-7} M. The figures are expressed as percentages of the data for the control lenses.

The results of measurements of water content in lenses incubated in dexamethasone or ouabain containing media are shown in Table III. Fresh lenses had a water content of 69 per cent – a figure consistent with the findings of previous investigators (Harris & Gruber 1962). Lenses incubated in control media had approximately the same water content (69%) while those incubated in dexamethasone or ouabain containing media (1×10^{-4} M) showed sharp increases to 73 and 73 per cent respectively.

Ion content of lenses incubated in varying concentrations of dexamethasone or ouabain are shown in Table IV. Fresh controls show a Na^+ concentration of 18 and K^+ concentration of 130 mEq/l. Three day control lenses show some increase in Na^+ and K^+ loss. However lenses incubated in dexamethasone show a great increase in Na^+ and a decrease in K^+. Lenses incubated in varying concentrations of ouabain show changes in ion content similar to those observed with dexamethasone.

Table II

Na^+ K^+ ATPase activity in lenses incubated in dexamethasone or ouabain (% of control)

Concentration	Control	Dexamethasone	Ouabain
1×10^{-3} M	100	10 ± 1 (21)	18 ± 5 (11)
1×10^{-4} M	100	24 ± 2 (38)	26 ± [illegible]
1×10^{-5} M	100	33 ± 2 (21)	[illegible] ± 3 (11)
1×10^{-6} M	100	46 ± 3 (18)	5[illegible] ± 3 (18)
1×10^{-7} M	100	74 ± 5 (13)	90 ± 7 (13)

Numbers in brackets represent number of lenses in each experiment

Table III
Water content of lenses incubated for 4 days (% of total weight)

Control (fresh)	69 ± 0.7	(20)
Control (incubated)	69 ± 2	(8)
Dexamethasone (1×10^{-4} M)	75 ± 1*	(8)
Ouabain (1×10^{-4} M)	73 ± 1	(8)

Numbers in brackets represent number of lenses in each experiment
* $P = < 0.01$ ** $P = < 0.025$

Table IV
Ion content after 3-day incubation (mEg/L)

Control (fresh) (17)		Na^+ 18 ± 0.2		K^+ 130 ± 0.7	
	10^{-4}		10^{-5}		10^{-6}	

	10^{-4} Na^+	10^{-4} K^+	10^{-5} Na	10^{-5} K^+	10^{-6} Na^+	10^{-6} K^+
Control (incubated)	24 ± 5 (10)	122 ± 2 (11)	24 ± 5 (11)	120 ± 2 (11)	25 ± 1 (9)	123 ± 0.5 (9)
Dexamethasone	84 ± 3 (26)	66 ± 10 (26)	62 ± 11 (12)	87 ± 15 (12)	65 ± 4 (9)	80 ± 1 (9)
Ouabain	83 ± 12 (26)	60 ± 9 (26)	68 ± 29 (12)	83 ± 28 (12)	62 ± 19 (9)	89 ± 19 (9)

Discussion

A number of authors including Kinoshita (1974) and Kinsey (1965) have commented on the importance of the cation pump mechanism in maintaining the viability and normal transparency of the lens. Since the Na^+ K^+ ATP'ase is thought to be an important component of the pump, interference with this enzyme would lead to a breakdown of the normal pump-leak balance. In the case of the specific inhibitor of Na^+ K^+ ATP'ase ouabain and in the Nakano strain of mice inhibition of the enzyme has been found to be associated with cataract formation.

The data presented in this communication show that calf lenses incubated for

three days in media containing ouabain or dexamethasone undergo cataractous changed which are accompanied by a marked decrease in Na^+ K^+ ATPase. Serial measurements of enzyme activity show a gradual decrease which correlates well with the decrease in light transmission noted over the same period of incubation. It is also shown that with both dexamethasone and ouabain there is a pharmacologic effect since a concentration of 1×10^{-3} M causes greater inhibition of enzyme activity than 1×10^{-7} M

Previous work by Harris & Becker (1965) has shown a loss of K^+ and an increase in Na^+ in rabbit lenses incubated in prednisolone phosphate 1×10^{-3} M at 0 and 37 C More recently Friedburg et al (1974) have shown *in vivo* an increase in Na^+ concentration in the lens when methyl prednisolone was injected into the vitreous of guinea pigs These changes would be consistent with a reduction in the activity of the cation pump and would suggest an inhibition of the Na K ATPase of the lens It is of interest therefore that our experiments showing an inhibition of the enzyme also demonstrate an increase in Na^+ and a loss of K ion. The present work appears to confirm our original postulate namely that dexamethasone might exert an inhibiting effect on the cation pump

The concentrations of dexamethasone used in these experiments are in the range of 1×10^{-3} M to 1×10^{-7} M It may be argued that concentrations of 1×10^{-3} M or 1×10^{-4} M are a magnitude or two higher than would be expected at physiologic or pharmacologic levels However since similar effects may be seen in our *in vivo* experiments at concentrations of 1×10^{-5} M $- 1 \times 10^{-7}$ M it would suggest that these observations may have physiologic significance Moreover in an experimental study designed to assess the amounts of steroid delivered to the eye by various vehicles Green & Downs (1974) found that the concentrations of prednisolone phosphate administered to the cornea of rabbits was of a similar order of magnitude ($1-4 \times 10^{-5}$ M) used in some of our studies

Other effects of steroids on the lens are of course also possible It is known that dexamethasone interferes with glucose uptake into lymphosarcoma P1798 cells but hexokinase is not inhibited (Rosen et al 1970) The mechanisms involved in this process are at present obscure and a membrane effect is thought to be involved. In addition steroids are known to induce certain enzymes such as tyrosine amino transferase (Gerschenson et al 1970 Cranner et al 1970) and phenylethanolamine N methyl transferase (PNMT) (Pohorecky et al 1970) in certain tissues such as liver fat cells and adrenal gland The effect of steroids such as aldosterone on RNA metabolism and protein induction (RNA polymerase) are also well known (Fraser 1971) It is not known at this point whether these effects are related to the work reported here

Clinical experience has shown that steroids such as dexamethasone 21 phosphate have an important cataractogenic effect The mechanisms involved have been

obscure In the preceding communication it was shown that a steroid induced cataract could be experimentally produced It was postulated that inhibition of Na+ K+ ATPase might be an important mechanism involved in this process The present work shows that a significant inhibition does in fact occur under these experimental conditions Observations on ion concentrations and water content further suggest that inhibition of the cation pump may play a significant role in the formation of steroid cataract *in vitro*

Acknowledgments

The authors would like to thank Dr Leo T Chylack for his helpful advice in the conduct of this study We would also like to express our gratitude to Professor George Benedek and Dr Jin Kinoshita for their comments and suggestions during the course of this work.

References

Behari M & Grossman B J (1968) Posterior subcapsular cataracts related to long term corticosteroid treatment in children *Amer J Dis Child 116* 604

Benatti C & Ball F (1971) Cataract caused by long term corticosteroid therapy and its prevention. *Minerva Pediat 23* 1590

Bonting S L (1965) Na+ K+ activated adenosine triphosphatase and active cation transport in the lens In Harris E J ed *Symposium on the Lens* p 347 C V Mosby Co St. Louis

Bonting S L Caravaggio L L Hawkins N M (1963) Studies on sodium-potassium activated adenosine triphosphatase VI its role in cation transport in the lens of cat calf and rabbit *A ch. Biochem 101* 47

Braver D A Richards R D & Good T A (1966) Posterior subcapsular cataract in corticosteroid treated children *J Pediat 69* 735

Fraser R (1971) The effect of steroids on the transport of electrolytes through membranes In Smellie R M S ed *The Biochemistry of Steroid Hormone Action*, p 101 Academic Press London

Friedburg D Kroner H & Rosenstell L (1974) *In vivo* action of corticosteroids on the guinea pig lens *Ophthal Res 61* 131

Gerschenson L. E. Anderson M Molson J & Okigake T (1970) Tyrosine transaminase induction by dexamethasone in a new rat liver cell line *Science 1 0* 859

Granner D K Thompson E. B & Tomkins G M (1970) Dexamethasone phosphate induced synthesis of tyrosine aminotransferase in hepatoma tissue culture cells *J Biol. Chem 245* 1472

Green K & Downs S J (1974) Prednisolone phosphate penetration into and through the cornea. *Invest Ophthal. 13* 316

Gullberg R & Elman A (1973) Posterior subcapsular cataract in rheumatoid arthritis patients after long term paramethasone therapy *Scand. J Rheumat. 2* 113

Harris J E & Becker B (1965) Cation transport of the lens *Invest Ophthal. 4* 709

Harris J E & Gruber L (1962) The electrolyte and water balance of the lens. *Exp Eye Res. 1* 372

Iwata S & Kinoshita J H (1971) Mechanism of development of hereditary cataract in mice *Invest Ophthal 10* 504

Kennedy T (1970) Cortisone induced opacities of the crystalline lens *Trans Ind Cn Ophthal 2* 28

Kinoshita J H (1974) Mechanisms initiating cataract formation *Invest Ophthal 13* 13

Kinsey V E (1965) The pump leak concept of transport in the ocular lens. In Rohen J W ed *The Structures of the Eye* p 383 F K Shattauer Verlag Stuttgart

Kristensen P (1968) Posterior subcapsular cataract and systemic steroid therapy *Acta ophthal (Kbh) 46* 1025

Loredo A Rodriguez R O & Murillo L (1972) Cataract after short term corticosteroid treatment *New Engl J Med 286* 180

Lowry O H & Lopez J A (1946) The determination of inorganic phosphate in the presence of phosphate esters *J Biol Chem 162* 421

Lowry O H Rosebrough N J Farr A L & Randall R J (1951) Protein measurement with Folin phenol reagent *J Biol Chem 193* 265

Mayman C I (1972) Inhibitory effect of dexamethasone on sodium potassium activated adenosine triphosphatase of choroid plexus in cat and rabbit *Fed Proc 31* (2) 591 Abs

Mayman C I (1974) Developmental aspects of sodium potassium activated adenosine triphosphatase (Na^+ K^+ ATP ase) in choroid plexus *Neurology (Minneap) 24* 3 0

Mayman C I & Tyerina M L (1979) Effect of dexamethasone on sodium potassium activated adenosine triphosphatase of choroid plexus (Submitted for publication)

Miller D Tyerina M L & Mayman C I (1979) *In vitro* production of steroid cataract in bovine lens *Acta ophthal (Kbh) 57* 1101

Pohorecky L A Piezzi R S & Wurtman R J (1970) Steroid induction of phenylethanolamine N methyl transferase in adrenomedullary explants independence of adrenal innervation *Endocrinology 86* 1466

Rosen J M Millholland R J & Rosen F (1970) A comparison of the effect of glucocorticoid on glucose uptake and hexokinase activity in lymphosarcoma P1798 *Biochim biophys Acta 219* 447

Samson F E Jr & Quinn D J (1967) Na^+ K^+ activated ATPase in rat brain development *J Neurochem 14* 421

Taub S J (1968) The incidence of cataracts in steroid treated children *Eye Ear Nose Thr Mon 77* 133

Author's address

Chaim I Mayman M D F R C P (C) Department of Neurology Harvard Medical School Beth Israel Hospital 330 Brookline Avenue Boston MA 02215 USA

JUDICIA DE NOVIS LIBRIS

Documenta ophthalmologica Proceedings Series Volume 18 Progress in Anterior Eye Segment Research and Practice Dr W Junk b Publishers The Hague Boston London 19 9 Edited by O Hockwin & W B Rathbun 3"0 pages Price US dollar 75 –

Volume 18 in th Documenta Ophthalmologica Proceedings Series is in honour of Prof John E Harris Unive sity of Minnesota and consists of submissions from invited authors working with anterior e segment research and ractice The volume is an excellent compilation of current concep s in this field of ophthalm logy and includes the latest results from a great number of outs anding workers It is to be rec mmended

Preben Gilbert

Wulf Ehrich Atlas der Kontaktlinsen A and Enke Verlag Stuttgart 19/9 169 pages 445 illustrations 19 –

Contact lens fitting is a practica k onstrations of patients or clinical phothographs can often tell m pret e descriptions Contain ng more illustrations than pages this k tc tled atlas. The text is given as legends to pictures or tables The aut t w his lectu e so richly supported by slides in a conversational form Case h n repl systematic exposition Few aspects of this facetted subject are omitted ut pc lymer chemistry the physical chemical and optical properties of the basic isati n and desinfection and product control

Further information is gi en t e and des gn examinations necessary prior to fitting techniques for soft lense CAB l e Silic ne lenses and PMMA lenses Special consideration is given to bandage lenses and iris lenses

The book is recommended to e erybody interested in contact lens practice

V Dreyer

Dav d J Apple and Mauric F Rabb Clin copathologic correlation of ocular disease a text and stereoscopic atlas 2nd ed 5 6 pages 8 illustrations and 112 stereoscopic views in colour on 16 View Master reels Mosby St Louis 1978 Price US d llar 138 75

This new edition has been expanded with approximately 400 new clinical photographs and photomicrographs and there are 50 new colour stereophotographs The atlas correlates excellently the cl nical and pathological find ngs in ocular diseases and the many illustrations are most instructi e

The book is to be warml recommended to all student c phthalmologists Every ophthalmic department ought to have an example in its library

A View Master is a ailable in any toyshop – Dkr 32 –

S Ry Andersen

Bojan Ranko Vitamin A und Auge Abhandlungen aus dem Gebiete der Augenheilkunde Sammlung von Monographien Band 47 Georg Thieme Leipzig 19 9 0 pages, 13 illustrations and 4 tables Price DM 19 80

Vitamin A a vital food constituent for man and for animals was identified at the beginning of this century In spite of extensive research and a large scale production of this vitamin much disease is still due to lack of vitamin A Figures of 100 000 new blind children every year because of A avitaminosis are mentioned and in some countries e g India, the number of affected persons is huge A presentation and discussion of the ophthalmologic problems related to lack of vitamin A and to overdoses of vitamin A is therefore still absolutely relevant

The author gives a survey of vitamin A occurrences in food and in organisms and its metabolism The main section of the book is a presentation of the ophthalmological symptoms of vitamin A deficiency This is very useful The chapter on vitamin A in ophthalmological practice hardly stands to a scientifical criticism e g it is claimed that retinitis pigmentosa may be effectively treated by vitamin A (!) The final chapter on hypervitaminosis A is quite short Chronic as well as acute intoxication is discussed, but the possible occurrence of papilloedema is not mentioned

The book would seen to be of interest to those particularly engaged in the treatment of avitaminosis A or in research on eye complications in vitamin A deficiency

Nel Ehers

J M Emery Current concepts on cataract surgery Selected proceedings of the fifth biennial cataract surgical congress C V Mosby Comp St Louis 1978

These proceedings include 197 brief and concise articles on almost every aspect of cataract surgery All the major headlines are wellknown intracapsular extraction extracapsular extraction intraocular lenses operative complications postoperative complications, correction for aphakia and results of cataract surgery

It may be worthwhile to mention a few of the topics The discussions on outpatient surgery are interesting The choice of surgical procedure may be determined by several factors, but in the northern european countries there would seem to be severe economical arguments in favour of outpatient cataract surgery Other topics discussed are phacoemulsification and intraocular lens implants Techniques are still under development and the long term fate of the corneal endothelium after these procedures still await elucidation

There can be little doubt that for a practically working ophthalmologist it would be extremely useful to participate in such a congress and the participant may afterwards, in the proceedings look up any particular matter that he might want to recall However the viewer cannot keep back the impression that the report will be of a limited benefit for the unprepared readers as well as for the writers and that the real interest in the publication of these congress proceedings is on the part of the publishing companies. Regular reading of a few of the more important ophthalmic journals would seem to give the same amount of information

With this criticism the present volume may still be found useful due to its discussions which take up a substantial part of the pages

Nel Ehers

Rudolf Sachsenweger: Kompendium und Atlas der Augenheilkunde. Gustav Fischer Verlag, Stuttgart, New York. 179 pages, 278 figures.

The author's intentions – to facilitate, intensify and rationalize the teaching of medical students in the growing field of ophthalmology – have been completed by limiting the content to a level which corresponds to the knowledge required from Danish medical students. Furthermore, the text is short though sufficient and comprehensible. Though the text is compressed and the letters are small, a good layout enables the students to review the book quickly. Recapitulations and small tables have the same purposes.

This book should be considered in the training program for medical students.

Tor Søer

Georg Eisner: Ophthalmologische Operationsindikationen. Eine Orientierung für den Allgemeinpraktiker. Verlag Hans Huber, Bern, Stuttgart, Wien 1979. 81 pages, 80 illustrations. Price Fr 68.– DM 76.–

This is a manual for the general practitioner who wishes to obtain a summary insight into the principles of modern ophthalmic surgery as far as indications, procedures and consequences are concerned. To the general practitioner the section on photocoagulation in diabetic retinopathy may be of particular interest. Recent advances such as vitrectomy, corneal transplantation, Cuppers Faden operation in nystagmus and incomitant squint are probably dealt with in too much detail for the general practitioner. On the other hand, such every day ophthalmological problems as cataract are mentioned to briefly (e.g. nothing about corneal incisions or indications for or difficulties in intraocular lens implantation, too little about the problems about combined cataract and glaucoma operations). Nothing is stated about the surgical treatment of concomitant squint.

Thus, in many respects, the book is a valuable guide for the general practitioner in countries where it is difficult for the patient to contact an ophthalmologist. Maybe the book might also be of interest to practising ophthalmologists who have not been in touch with ophthalmological surgical practice for the past 10–15 years or more.

Anders Bruun Laursen

Arthur Sieu Ming Lim and Ian J. Constable: Colour Atlas of Ophthalmology. H. Kimpton, London 1979. 151 pages, 193 illustrations (189 in colour). Price Eng. pds 6.50.

A small handy book with handsome well-chosen instructive colour photographs on matt paper. Most eye diseases are represented, several patients are exotic (one of the authors comes from Singapore). An amusing picture shows a Chinese superstition: Counter irritant placed on the forehead above an eye with acute glaucoma.

The text is concise and like a compendium. It is instructive and up-to-date. The reviewer disagrees with the authors on only one or two points. There are few misprints (Scotometer and Goldman perimeter figures are reversed, pilocarpine 6%).

The book is suitable as additional reading for students, ophthalmological assistants and nurses.

Mogens Norn

The International Society for Clinical Electrophysiology of Vision (ISCEV)

will hold its XVIIIth Symposium in Amsterdam from 18th to 22nd May 1980

The three main subjects of the symposium are
a) peripheral processing emphasizing on pathology and physiology
b) neural pathways e g routing recording from subcortical structures
c) central processing especially contributions on binocularity and pathology will be appreciated

The symposium language will be English Offers of papers are kindly requested there will be facilities for scientific demonstrations and posters

For further information and application forms please write to
Professor H Spekreijse Netherlands Ophthalmic Research Institute
P O Box 6411 1005 EK Amsterdam The Netherlands

Consilium Europaeum Strabismi Studio Deditum

The XIth Meeting of the CESSD will take place at The Institute of Child Health Guilford Street London WC1 on April 18th and 19th 1980 Prior to the European Ophthalmological Congress in Brighton

Enquiries and information may be obtained from Miss Barbara M Lee MBE Moorfields Eye Hospital High Holborn London WC1 7AN

(Cont. from cover page 2)
author(s) The m nus s gn should be shown as – In decimal fract ons use a full stop and not a comma. Footnotes should be avoided If essential place at the bot om of the respective p ge and number them concecutively In abbreviations and symbols only standardized terms should be used Un amiliar abbreviations must be defined when first used

ILLUSTRATIONS All photographs drawings and graphs are referred to as figures abbreviated (Fig) and should be numbered in sequence with Arabic numerals. Figures with legends should always be planned to f t the printed column 117 × 190 mm and the preferred place of each figure marked in the left margin of the manuscript. Indicate number orientation author's name and title of article on the back in soft pencil Each figure should have a legend

Photographs, except of microscope preparations, should be about one and a half to two times the size of the final block and must be unmounted glossy enlargements showing good detail and moderate but not excessive contrast. Authors should see that extraneous areas are omitted Negatives and slides cannot be used Labels arrows etc should be professionally prepared lettering uniform and large enough to be read easily when reduced Photographs of microscope preparations should be carefully prepared to fit the printed column in unaltered size according to the magn fication mentioned in the legend and text.

Line drawings should be drawn with black India ink on white paper and should be about twice the size of the finished block.

Graphs should be plotted on plain white or blue squared paper and be about twice the size of the f nished block grid lines that are to show in the engravings should be inked in black. Prominence of lettering lines curves and s gns should correspond to the relative importance of each component and should be easy to read Costs of colour illustrations must be borne by the author

AUTHOR S ADDRESS List after references the exact postal address of the author to whom communica ons should be sent.

REFERENCES. The author(s) is responsible for accurate references References in the text should quote the last name(s) of the author(s) and the year of pub ication Brown & Smith (1968) or Brown et al (1968) when there are three or more authors The reference list should include only those publ cations mentioned in the text and listed in alphabetic order Titles of journals should be abbreviated according to World Medical Periodicals

Examples

Be ker B & Sh ffe R. N (1965) *Diagno is and Ther py of the Glaucomas* p 328 2nd d. Mosby St. Louis.
H ll we F C & Gr ham P A. (1966) The F rndale glaucoma survey In Hunt, L. B., ed. *Glauc ma Symposium* pp 24-44 Liv gstone Ltd. Lo d n.
Rise, D & Sim nsen, S E. (1969) Intraocular p ssure in unilateral opti erve les on. *A ta ophthal (Kbh)* 47 750-756

TABLES should be numbered consecutively in Roman numerals and cited in the text. The approximate location in the text should be indica ed in the left margin of the manuscript. Each table shou d be typed on a separate sheet and have a le end which makes its general mean ng comprehens b e without reference to the text.

LEGENDS. Capt ons to figures should be typed on a separa e sheet and numbered correspondingly They should make the meaning of each illustration understandable without reference to the text.

PROOFS will be sent to one author only Any alterations made during linguistic ed ting by the publisher should be checked carefully and the proof returned w th the least possible delay and w th the minimum of essential corrections Authors w ll be cha ed for any major alterations and excess ve changes may be disallowed If a sign f cant amount of new mate ial is added the paper may be redated. Second proofs will be sent only if specif cally and justif ably requested by the author Final proofs are read by the publisher

REPRINTS. F fty reprints without cover will be s ppl ed free of charge for each article Additional reprints can be suppl ed if ordered on the reprint order form which will accompany the proofs.

SHORT COMMUNICATIONS not exceed ng one or two printed pa es are acceptable if the findings are of utmost importance and require a rap d publication.

SUPPLEMENTS. Larger papers may be publ shed as supplements of the author's cost. After acceptance supplements are not subject to ed tor al revision. Further information can be obtained from the publishers

---

Bus ness communications including subscriptions should be sent to the publishers

Scriptor Publisher ApS 15 Gasværksvej DK 1656 Copenhagen V Denmark

SUBSCRIPTION PRICE per volume of 6 issues, published bimonthly D kr 460 plus postage D.kr 50